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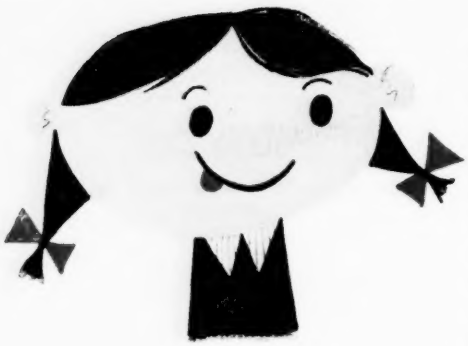

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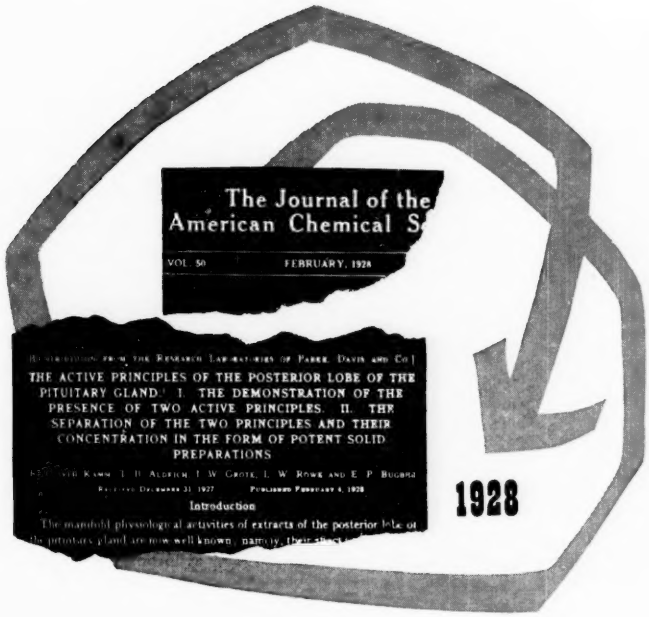
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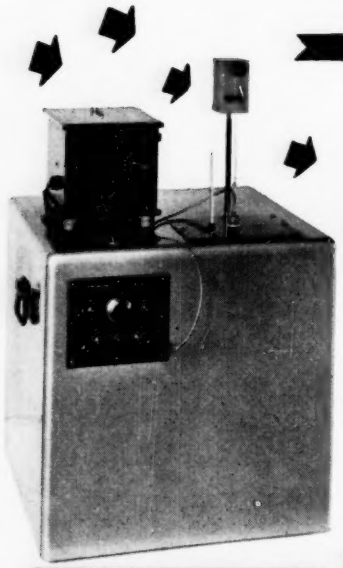

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General Malaise	10	8
Cough	7	6
Dyspnea	13	8
Headache	6	6
Nausea	6	4
Vomiting	4	3
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Escher, G. C., et al.: Clinical Research Proceedings 1:51 (Apr.) 1953.

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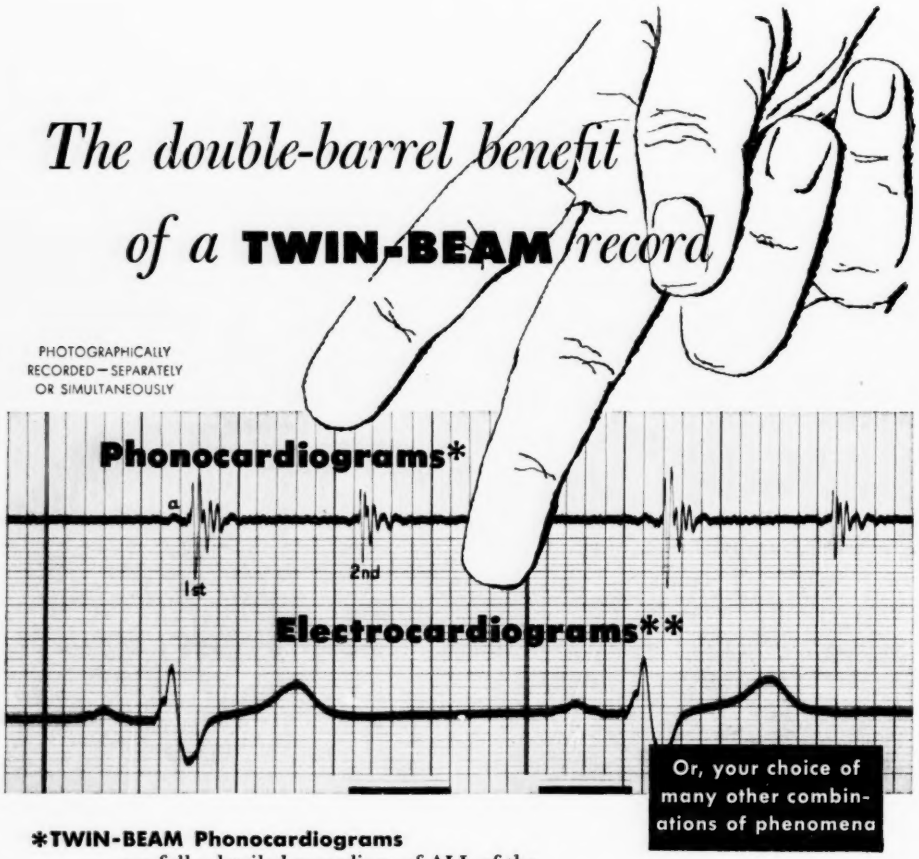

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EPISODIC STUPOR ASSOCIATED WITH AN ECK FISTULA IN THE HUMAN WITH PARTICULAR REFERENCE TO THE METABOLISM OF AMMONIA [1, 2]

By WILLIAM V. McDERMOTT, JR. AND RAYMOND D. ADAMS

(From the Surgical and Neurological Services, Massachusetts General Hospital, and the Departments of Surgery and Neurology, Harvard Medical School, Boston, Mass.)

(Submitted for publication June 16, 1953; accepted September 1, 1953)

The effect on animals of ligating the portal vein and diverting the blood from the portal system directly into the inferior vena cava through a surgically constructed fistula was first studied by Eck in 1877 (1). As a mark of respect for the ingenuity of these experiments, the eponym, "Eck fistula," has been perpetuated in medical writings. Animals so treated can be made to survive for long periods of time in apparently good health and therefore the Eck fistula has been used extensively as a means of investigating the metabolic activities of the liver. Although dogs with an Eck fistula may remain in good clinical condition, it has been demonstrated that the liver undergoes a certain amount of atrophy; the production of plasma proteins, hemoglobin, and bile pigments is diminished; serum lipid levels are low despite adequate fat absorption; and the consumption and urinary output of water are increased (2–5). Perhaps the most interesting and controversial observation derived from these experiments has been the phenomenon of "meat intoxication," a neurological disorder consisting of stupor, ataxia, convulsions, and finally coma, which occurs in Eck fistula dogs either after the ingestion of meat, or spontaneously. This phenomenon has been studied extensively (6–10) and, while it has been suggested that various degradation products of protein metabolism are responsible, the pathogenesis actually remains obscure.

Construction of an anastomosis between the portal vein and the inferior vena cava with or without interruption of the portal vein is frequently carried out in patients with portal hypertension for the treatment of esophageal varices. This anastomosis undoubtedly reduces the pressure within the portal system and some portal blood flows through the new channel directly into the inferior vena cava. Nevertheless, the presence of pre-existing obstruction to the portal flow, either intrahepatic or extrahepatic, almost invariably has favored the development of an extensive collateral circulation, and blood may reach the liver through the hepatopedal channels described by Edwards (11). Therefore, the conditions of a true Eck fistula are not reproduced.

Recently, in a modification of existing surgical methods for the treatment of cancer of the head of the pancreas one of us (W. V. M.) (12) described the techniques which make it feasible to resect the portal vein and to construct an anastomosis between the superior mesenteric vein and the inferior vena cava. So far as is known, the performance of this procedure has resulted in the first Eck fistula to be established in man in the absence of pre-existing portal hypertension and collateral venous circulation. The case which was described in the above report offered an unusual opportunity to study the physiologic effects of this surgical anomaly and for this reason a number of metabolic studies were instituted. The findings of these studies are not presented here in detail because their significance was overshadowed by the extraordinary clinical course of the patient, *i.e.*, the occurrence of episodic stupor. This neurologic disorder was transient, completely reversible and unaccompanied by any demonstrable biochemical change other than intermittent, precipitous elevations of the concentration of ammonia in the blood.

CASE REPORT

A. S. (MGH No. 752571), a 69 year-old retired Italian laborer, entered the Massachusetts General Hospital on September 25, 1951 with jaundice of several weeks' dura-

[1] The biochemical studies were supported by the Atomic Energy Commission and the Damon Runyon Fund.

[2] The expense of the neurological studies was defrayed by grant No. B36 from the U. S. Public Health Service.

tion. The clinical and laboratory findings pointed to an obstructive origin of the jaundice, and on October 8, 1951, an exploratory laparotomy was performed. A hard, nodular cancer measuring 5.0 cm. in greatest diameter was found in the head of the pancreas. The tumor invaded the superior mesenteric vein near its junction with the splenic vein and in order to perform a pancreatoduodenectomy it was necessary to resect the portal vein. The end of the superior mesenteric vein was then joined to the side of the inferior vena cava thus establishing a true Eck fistula. No distant metastases were seen, and except for bile stasis the liver appeared normal by gross examination.

Postoperatively, the jaundice rapidly subsided and the immediate clinical course was uneventful. The patient was discharged from the hospital on the nineteenth postoperative day. Five weeks after operation he was readmitted in order that metabolic studies could be carried out and at this time he was in excellent health. On the fourth day in the hospital he became irrational and disoriented and was incontinent of urine. This episode, which progressed to stupor, developed over a period of several hours and lasted two or three days after which mental function then returned to normal. One month later, while still in the hospital, these symptoms recurred and this time the stupor progressed to coma. There were no focal neurological signs. Again spontaneous recovery took place. A third episode, identical in type to the others, occurred after a further interval of three weeks. It was the opinion of the attending physicians and consultants that these episodes of stupor or coma represented attacks of apoplexy and it was so stated in the original report of the case (12).

After he had recovered completely from this third episode, the patient returned to his home and remained entirely asymptomatic until March 25, 1952. Once again he became confused and then comatose and was readmitted in this state. As in previous attacks there were no focal neurological signs and cardiac and renal functions remained normal. The function of the liver had not been greatly altered as judged by standard laboratory tests (Table I). There had been evidence of a mild impairment of carbohydrate and fat metabolism immediately after the operation, as revealed by tests of glucose tolerance and fat absorption, but not of a degree sufficient to account for a disturbance of nervous function of this severity. A total nitrogen balance study, including measurements of non-protein nitrogen, urea nitrogen, creatine and creatinine, demonstrated no abnormalities.

Despite the lack of laboratory evidence of any gross physiological abnormality, it became obvious that these episodic disturbances of consciousness were stereotyped in pattern and could only represent the effect of some unknown metabolic disorder on the nervous system. Consequently, it was decided to define the nature of the neurological syndrome by clinical and electroencephalographic methods and to search for possible clues as to the underlying biochemical abnormality.

METHODS

In order to obtain a more precise evaluation of the clinical status of the patient for purposes of correlation with electroencephalographic and biochemical data, the degrees of impairment of mental function were graded on a scale of zero to four. Normal mental function was recorded as zero. Coma, meaning a complete lack of awareness of any externally applied stimulus, was graded as

TABLE I

Preoperative and postoperative chemistries relative to liver function

Date 1951	Vandenbergh *mg. %* direct/indirect	Cephalin flocculation 24°	Cephalin flocculation 48°	Thymol turbidity *units* %	Alkaline p'tase Bodansky *units* %	Urobilinogen urine Ehrlich units	Prothrombin time % of normal	Total protein *Gm.* %	A/G ratio	Bromsulfalein % retention
Sept. 25	9.5/11.8	0	0	—	18.3	0.6	—	6.9	1.8	—
Oct. 3	—	—	—	—	—	0.4	—	—	—	—
Oct. 8	—	Operation		—	—	—	—	—	—	—
Oct. 9	10.4/14.4	—	—	—	—	—	—	—	—	—
Oct. 11	8.8/12.8	0	1+	—	8.4	—	75%	4.4	1.1	—
Oct. 14	7.7/11.0	—	—	—	—	37.1	75%	—	—	—
Oct. 16	7.2/10.4	0	1+	0.5	—	—	—	4.9	1.4	—
Oct. 25	3.4/5.0	—	—	—	—	2.2	—	6.1	1.1	21%
Nov. 13	—	—	—	—	—	3.6	—	—	—	—
Nov. 19	2.0/2.5	1+	2+	1.3	—	—	65%	6.0	1.3	12%
Nov. 20	—	—	—	—	—	—	57%	—	—	—
Nov. 26	—	—	—	—	4.7	0.9	—	—	—	—
Nov. 27	1.4/1.5	—	—	—	—	—	—	—	—	—
Dec. 3	1.0/1.0	—	—	1.3	—	0.2	45%	—	—	6%
Dec. 11	—	1+	1+	—	—	0.2	57%	5.0	1.1	—
1952										
Mar. 4	0.5/0.9	2+	2+	0.5	7.7	—	60%	4.9	1.2	—
Apr. 3	1.1/1.4	1+	3+	—	7.2	—	70%	6.9	1.4	—
May 13	1.1/1.4	1+	2+	—	5.8	—	—	5.8	1.2	—
June 13	0.7/1.1	1+	2+	—	7.3	—	—	5.9	1.4	—

TABLE II

The percentage recovery of ammonia nitrogen from standard solutions by the method employed in these investigations, and the range of normal blood determinations measured in healthy human controls

Determinations	$(NH_4)_2SO_4$ solutions containing: 0.198 μg. N/cc.	Per cent recovery	0.495 μg. N/cc.	Per cent recovery	1.97 μg. N/cc.	Per cent recovery	Normal blood determinations
1	0.193	97.5	0.500	101.	1.94	99.5	1) 58.9 μg. %
2	0.201	102.	0.512	103.	1.97	100.	2) 65.2 μg. %
3	0.213	108.	0.500	101.	1.94	99.5	3) 78.8 μg. %
4	0.189	95.4	0.462	93.3	1.92	97.5	4) 55.2 μg. %
5	0.163	82.3	0.479	96.8	1.97	100.	5) 49.9 μg. %
6	0.181	91.4	0.470	95.0	1.98	101.	6) 57.8 μg. %
7	0.201	102.	0.513	104.	1.97	100.	7) 57.8 μg. %
8	0.172	86.9	0.457	92.3	2.01	102.	8) 62.3 μg. %
9	0.193	97.5	0.489	98.8	2.03	103.	9) 71.0 μg. %
10	0.218	110.	0.489	98.8	2.00	102.	10) 50.8 μg. %
Average	0.192	96.9	0.487	98.4	1.97	100.	60.8 μg. % Average
Standard error	±.005		±.006		±.01		28.9 μg. % Range

four; whereas the slightest perceptible alteration of mental function, usually presenting as drowsiness, slowness of response, or incoherence of thought was rated as one. Severe mental confusion with disorientation for time and place, and incontinence of sphincters was recorded as two. In this state the patient talked loudly, wandered about the ward making nonsensical remarks, was often irritable and difficult to manage with a mood that vacillated from gaiety to truculence. Stupor, meaning a significant reduction in spontaneous activity with mental dullness, was graded three. In this latter condition the patient remained in bed or in a chair, was quiet most of the time and was incapable of answering any except the most simple questions regarding his personal identity. It was necessary to depend entirely upon assessment of the state of awareness and responsiveness because the only consistent neurological disorder was in this sphere. When deeply comatose the plantar reflexes were sometimes extensor but more often absent or flexor in type. Tendon reflexes were retained at all times as were pupillary, corneal, and pharyngeal reflexes. Fluctuating rigidity of the limbs, grasp and occasionally sucking reflexes could be evoked during confused and stuporous states. With practice, different observers were able to form similar judgments of the clinical state but it must be admitted that evaluation of the patient was always difficult because of his limited knowledge of English.

The electroencephalographic tracings were graded on a scale of zero, one, two, and three with zero standing for a normal record. The slightest recognizable degree of alteration from normal, rated one on our scale, consisted of some degree of disorganization of the regular wave forms and appearance of random slower waves (four to nine per second frequency) of higher voltage superimposed on a background of alpha waves. An absence of all normal waves and the occurrence of high voltage slow waves (*circa* two to five per second) were judged to be a three, the greatest degree of abnormality. A rating of two was given to those records which were intermediate, *i.e.*, moderately abnormal with a predominance of four to seven per second wave frequencies. In the more abnormal records there were occasional bursts of bilaterally synchronous, high voltage, slow waves of about two to three per second frequency. More often, however, all semblance of regularity of electrical activity had vanished.

Since elevated levels of blood ammonia had been demonstrated in dogs with Eck fistulas (6, 8) and neurological disorders induced in humans with liver disease by the administration of certain nitrogenous substances (13), it was decided to obtain quantitative measurements of blood ammonia levels from day to day during and between these episodes of stupor. A modification of the method devised by Conway (14) was employed. The accuracy and reliability of this modification and the range of values obtained in normal subjects are given in Table II. In brief, the technique was to obtain one milliliter of heparinized venous blood and introduce it immediately into the outer chamber of a diffusion dish containing one milliliter of saturated K_2CO_3. Diffusion was allowed to continue for ten minutes at the end of which time a titration of the contents of the inner chamber, which contained one per cent boric acid and an indicator, was carried out with 0.003 N hydrochloric acid. The amount of ammonia nitrogen in gamma per cent was then determined by calculating from the amount of .003 N hydrochloric acid required to titrate the contents of the inner chamber back to its original pH.

The patient remained on a ward in the hospital for a period of approximately six months. During the first part of this period, he was given a standard ward diet [3] and a record was kept of his protein intake. Base-line data concerning neurologic status, the electroencephalograms and blood ammonia levels were secured. Whenever there was the slightest derangement of consciousness, these procedures were repeated daily or as often as was deemed necessary to follow his status accurately. Frequent de-

[3] Standard ward diet consists of approximately 250 grams of carbohydrates, 80 grams of protein, and 90 grams of fat per day or 2,130 calories.

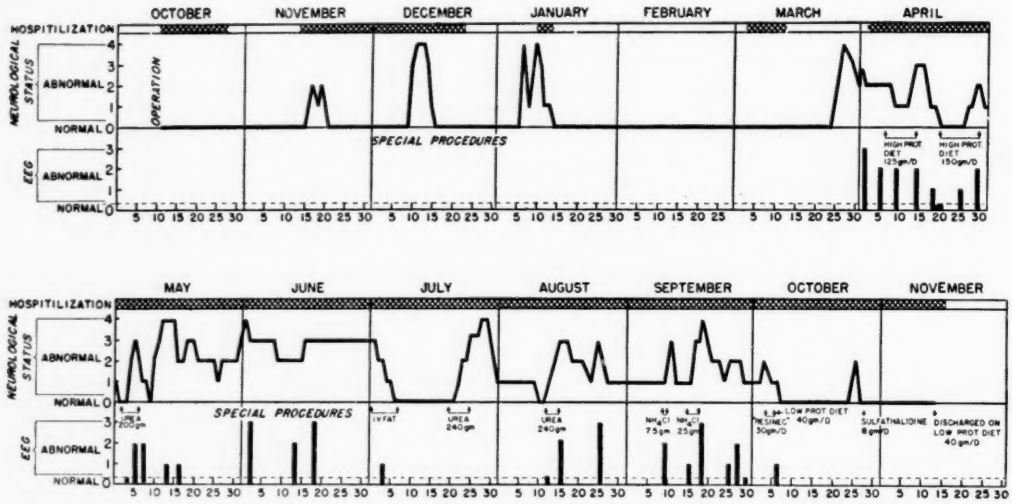

FIG. 1. DIAGRAMMATIC REPRESENTATION OF THE PATIENT'S CLINICAL COURSE, CORRELATING NEUROLOGICAL STATUS, ELECTROENCEPHALOGRAPHIC TRACINGS AND SPECIAL PROCEDURES WHICH APPARENTLY INFLUENCED THE CLINICAL COURSE

terminations of non-protein nitrogen, urea nitrogen, fasting blood sugar, and serum levels of sodium, potassium, and chloride were obtained during the early phases of the study, but as these remained consistently normal, they were later discontinued. The effects of a high protein diet and the oral administration of various nitrogenous substances were investigated.

RESULTS

In addition to the first four spontaneous episodes of confusion, stupor or coma previously mentioned in the case report, eight other episodes were observed during this long period of hospitalization. These latter were induced deliberately by the administration of excess protein, urea, ammonium chloride or "Resodec" (an ammonium and potassium cation exchange resin) in an attempt to discover the underlying cause of this abnormality. The clinical course of this patient's illness and the pertinent electroencephalographic data are graphically presented in Figure 1. Since the patient's future well-being depended on an understanding of the mechanisms causing this episodic stupor, it was essential to induce them in order to clarify the etiology of this condition and to obtain a basis for rational therapy.

It will be noted from the chart (Figure 1) that the first four episodes of stupor were brief and recovery was rapid (within two to four days) and complete. The only therapy was the parenteral administration of water, glucose, and electrolytes in adequate amounts. Later episodes were prolonged, lasting several days or even weeks at a time, with temporary improvement or worsening, often in relationship to certain specified procedures. The episode of May 10th to May 15th was induced by the oral administration of urea and was undoubtedly the most severe of any witnessed. For nearly a week the patient was in an alarming state of profound coma, manifesting no reaction to any externally applied stimuli. Even in this condition, however, there was no significant alteration of any of the measurable components of the circulating blood except as described below.

The electroencephalogram correlated well with the clinical status of the patient. The slightest mental aberration was always reflected in the electroencephalographic tracing. Moreover the degree of alteration of the electroencephalogram was roughly parallel to the clinical state. When the patient was slightly to moderately confused the electroencephalogram showed random slow waves (1 +); when stuporous all normal electrical activity was replaced by 4 to 7 per second slow waves (2 +). During deep coma there was a suppression of all normal electrical activity with only random delta waves remaining (3 +). With

the return of normal alertness and responsiveness, the electroencephalogram became normal. Bursts of bilaterally synchronous slow waves of high voltage were observed several times during severe confusion or stupor, when the electroencephalographic records were grossly abnormal.

A finding of special interest was the persistently elevated blood ammonia level even during periods when the patient was mentally clear and the electroencephalographic tracing was normal. In fact when compared with a series of patients on the ward who had neither liver disease nor an Eck fistula our patient's blood levels were always higher by fifty to seventy-five micrograms per cent.

The controlled metabolic study, during which an attempt was made to isolate some of the factors that could possibly be responsible for these episodes of stupor, was begun on April 6th. Since many investigators had reported that animals with Eck fistulae were intolerant of meat, developing "meat intoxication" after being fed liberal quantities of animal protein, it was planned at first to observe the effects of a high protein diet. It was difficult to maintain the proposed intake of one hundred and fifty grams of protein per day because after the first day the patient complained of feeling unwell and refused to eat this amount. An episode of stupor nevertheless occurred after three days on a relatively high protein diet. This experiment was not entirely satisfactory because he had not completely recovered from the episode of coma that brought him to the hospital on March 25th. The high protein diet was reinstituted on April 26th when the patient was mentally clear, and another episode of stupor occurred. Nitrogen balance studies were carried out during this episode but no abnormalities were detected. Dr. R. L. Berg studied the toxic products of proteolysis (8) and kindly analyzed samples of the patient's urine for these toxic substances but found none.

Urea was then given orally in repeated doses of 30 grams for four days. On the fourth day of the first trial of this drug, confusion and stupor developed, a degree of mental disorder that was graded as 3+. Urea was discontinued and although there seemed to be improvement for one day, this was followed by protracted coma which, as already stated, lasted for a week. During the administration of urea there was a slight rise in blood urea and non-protein nitrogen. No other biochemical abnormality was detected. After the

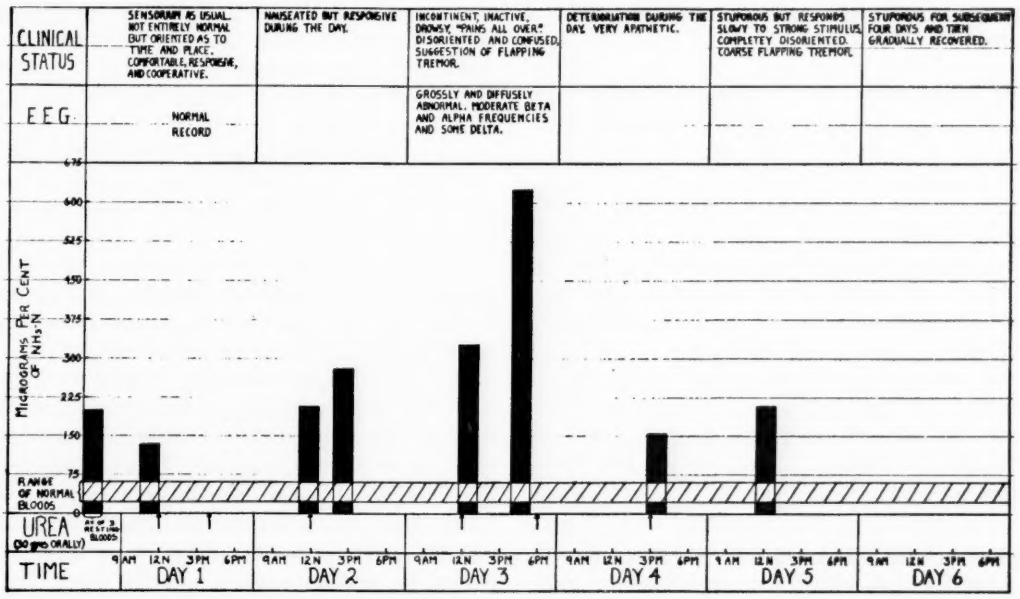

FIG. 2. THE EFFECT OF THE ORAL ADMINISTRATION OF UREA ON THE CLINICAL STATUS, THE ELECTROENCEPHALOGRAPHIC TRACINGS AND THE CONCENTRATION OF AMMONIA IN THE PERIPHERAL BLOOD

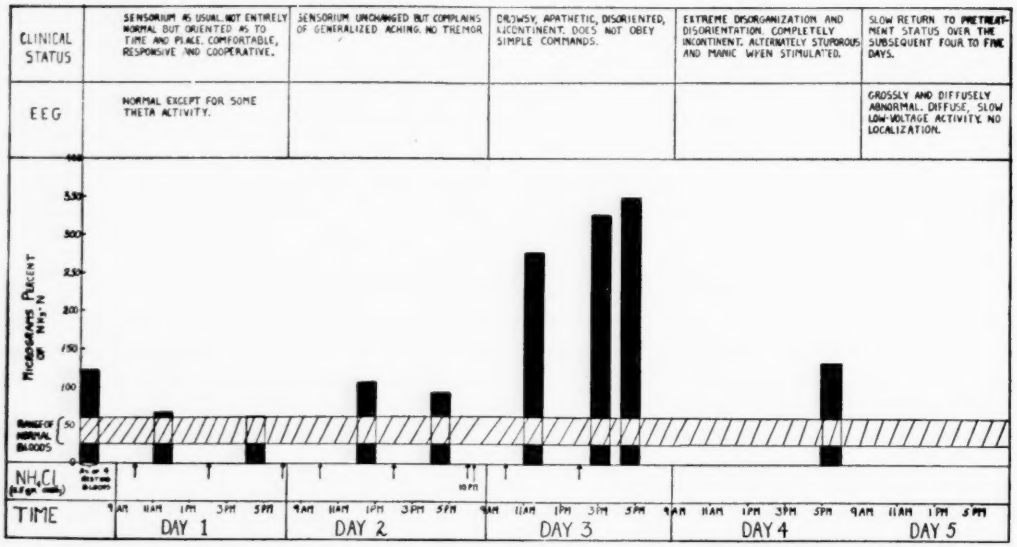
CLINICAL STATUS
SENSORIUM AS USUAL, NOT ENTIRELY NORMAL BUT ORIENTED AS TO TIME AND PLACE. COMFORTABLE, RESPONSIVE AND COOPERATIVE.
SENSORIUM UNCHANGED BUT COMPLAINS OF GENERALIZED ACHING. NO TREMOR
DROWSY, APATHETIC, DISORIENTED, INCONTINENT. DOES NOT OBEY SIMPLE COMMANDS.
EXTREME DISORGANIZATION AND DISORIENTATION. COMPLETELY INCONTINENT. ALTERNATELY STUPOROUS AND MANIC WHEN STIMULATED.
SLOW RETURN TO PRETREATMENT STATUS OVER THE SUBSEQUENT FOUR TO FIVE DAYS.
EEG
NORMAL EXCEPT FOR SOME THETA ACTIVITY.
GROSSLY AND DIFFUSELY ABNORMAL. DIFFUSE, SLOW LOW-VOLTAGE ACTIVITY. NO LOCALIZATION.
MICROGRAMS PERCENT OF NH3 N
RANGE OF NORMAL BLOODS
NH4Cl
TIME
DAY 1
DAY 2
DAY 3
DAY 4
DAY 5

FIG. 3. THE EFFECT OF THE ORAL ADMINISTRATION OF AMMONIUM CHLORIDE ON THE CLINICAL STATUS, THE ELECTROENCEPHALOGRAPHIC TRACINGS AND THE CONCENTRATION OF AMMONIA IN THE PERIPHERAL BLOOD

technique for analyzing blood ammonia had been perfected, this clinical experiment was cautiously repeated and the results are shown in Figure 2. After approximately 240 grams were given over a period of four days, he became confused and this progressed to stupor and coma despite immediate discontinuance of the drug. Concomitant with the clinical and electroencephalographic changes there was a sharp rise in the levels of ammonia in the blood. Improvement began after one day and recovery was complete within one week. This experiment was then repeated a third time with almost identical results. The repetition of these investigations seemed to be necessary in order to assure ourselves that these episodes of stupor were not coincidental with, rather than dependent on the administration of protein and urea.

It was decided next to study the effect of ammonium containing compounds. Ammonium chloride was administered in two and one-half gram doses to a total dosage of seven and one-half grams. This provoked only a mild and transient disturbance so the dosage was increased to a total of 25 grams in three days. The patient, who was slightly confused at the beginning of this experiment, became more so toward the end of the second day and on the third day was stuporous. The electroencephalographic abnormality became worse (3 +) and the levels of blood ammonia rose concomitantly (Figure 3).

"Resodec," a cation exchange resin, which liberates ammonia in the gastrointestinal tract was then tried. The effect was much the same as that produced by urea and ammonium chloride. After 30 grams were taken orally on two successive days, confusion increased in severity; the electroencephalographic abnormality became more pronounced; and the level of blood ammonia rose. When the resin was withheld, improvement occurred.

We were tempted to repeat many of these experiments and to undertake others which would confirm and elucidate certain obscure points in the pathogenesis of this episodic stupor. However, we had learned something about the mechanism of the clinical syndrome, and further study did not seem to be in the best interest of the patient. A therapeutic program designed to prevent the elevation of blood ammonia was then instituted. On a diet which allowed a maximum protein intake of not more than 40 grams per day, the patient has remained mentally clear for nearly four months except for two brief episodes of mild confusion lasting one to two days. He has been at

home and under the supervision of his family. As stated above, the level of blood ammonia during the asymptomatic periods has hovered around 150 micrograms per cent; but when oral sulfathaladine was given with this low protein diet, the ammonia level fell to slightly below 100 micrograms per cent, the upper limit of normal by this particular technique.

DISCUSSION

It is clear from these clinical data, even though based on but a single case, that a remarkable group of symptoms may be engendered by an Eck fistula in a human. This syndrome, which for convenience has been called episodic stupor, consisted of periods of impaired alertness and a retarded responsiveness which varied in degree from mild confusion to stupor or coma. Focal and lateralizing neurologic signs were absent but many of the accompaniments of confusion, stupor, or coma such as uninhibited and senseless behavior, fluctuating rigidity of the limbs, reflex grasping and sucking, and bilateral extensor plantar reflexes were observed. This episodic disturbance of mental function appeared to occur spontaneously without recognizable antecedent cause. The onset was acute; the course was progressive for one to several days; and recovery was either rapid, *i.e.,* within a day or two, or slower, within one or more weeks. Convulsions were not a part of the syndrome; at least they were never observed in the hospital though once the family reported a twiching of the limbs during stupor. Except for one severe and prolonged episode of coma, following which a slight degree of intellectual impairment persisted for several weeks, recovery was complete and there was no evidence of residual neurologic damage.

It is hardly valid to draw sweeping conclusions from a single case, yet it seems reasonably certain that a highly characteristic neurological syndrome has been induced in a human with an Eck fistula by a high protein intake, by urea, by ammonium chloride, and by an ion-exchange resin which liberates ammonia. The onset of these symptoms has been accompanied by a sharp rise in blood ammonia levels, so it would appear that the ingestion of substances which either contain ammonium ion or which during digestion may form ammonia in the gastrointestinal tract have an adverse effect on the central nervous system.

Although relatively little is known about the metabolism of ammonia in the human, there is a sufficient background of data from animal experiments to postulate a rational explanation for these observations. Ammonia is known to be a very toxic substance. Baldwin (15) has summarized the mechanisms by which several species eliminate ammonia, and concluded that "conversion of ammonia to other products is an indispensable adaptation to limitation of the availability of water." In the human, this adaptation takes the form of synthesis of urea in the liver, probably through the mechanism of the ornithine cycle as postulated by Krebs (16). Regardless of the details of this mechanism, it is well-established that an adequately functioning liver is essential in mammals for the elimination of ammonia (17). The kidneys are believed to have no part in the removal and, in fact, actually contribute to the total ammonia formed in the organism (18). All the sources of ammonia formation are not known but in addition to the processes of deamination within the body, ammonia is formed within the gastrointestinal tract by the action of urea-splitting micro-organisms and presumably is absorbed in relatively high concentrations into the portal system (19).

If the data from the investigation of mammals are applicable to the human, it is reasonable to suppose that, with an Eck fistula in which the blood in the portal system by-passes the liver, the organism is unable to detoxify the relatively large amounts of ammonia entering the portal system from the gastrointestinal tract with sufficient rapidity to prevent an elevation of ammonia concentration in the systemic circulation. This elevated level of ammonia may well interfere, directly or indirectly, with the metabolism of the central nervous system. The relationship between the neurological symptoms and ammonia levels in the blood is not an exact and reciprocal one, however. The onset of the syndrome of episodic stupor has been consistently correlated with a high blood ammonia level, but the blood level falls rapidly when the ingestion of ammonia containing compounds is discontinued whereas the central nervous system symptoms continue for some hours or days. It may be that the elevation in blood ammonia which was consistently

observed at the onset of symptoms, was only a reflection of an unknown disturbance in the complex mechanisms by which ammonia is metabolized in the body.

The neurologic syndrome in our case resembled "hepatic coma" in many respects. The rapid impairment of consciousness with attendant electroencephalographic abnormalities is common to both conditions. Inability to sustain the contraction of groups of muscles causing a flapping movement of the outstretched hands which has been found in a majority of cases of hepatic coma, was seen but infrequently. It may be recalled that Phillips, Schwartz, Gabuzda, and Davidson (13) were able to induce in patients with cirrhosis of the liver, a syndrome resembling "hepatic coma" by the oral administration of urea, ammonium chloride, diammonium citrate and "Resodec." If central nervous system symptoms can be precipitated by absorbed ammonia from the gastrointestinal tract by-passing the liver *via* an Eck fistula, it is reasonable to suppose that hepatocellular damage might interfere with the normal synthesis of ammonia to urea and have the same effect. In either instance there would be an impairment of certain of the metabolic activities of other organs, particularly the brain. It is also possible that shunting of blood directly from the portal to the caval system occurs in many cases of chronic liver disease, and that the factors operating in our case of human Eck fistula also play a role in the genesis of hepatic coma. This whole problem requires further study.

From the available evidence the conclusion that there was no serious degree of liver disease in our case is probably justified. Although the patient was jaundiced for several weeks before operation, direct inspection of the liver disclosed only bile stasis without atrophy or biliary cirrhosis. Also, repeated tests of hepatic function failed to demonstrate any consistent abnormalities. Considering, however, the acknowledged inadequacy of current tests of liver function and the changes which are known to occur in the liver of the dog after the construction of an Eck fistula, one must admit of the possibility that some degree of hepatic derangement did exist.

The relationship between this syndrome of episodic stupor in the human and "meat intoxication" in the dog also needs further clarification.

Finally attention should be called to the fact that the chemical disturbance attending an Eck fistula in humans deserves to be ranked with hypoglycemia, acidosis, uremia, hypokalemia, hyponatremia, and porphyria as a cause of episodic stupor or coma.

CONCLUSIONS

1. In a human with a surgical Eck fistula a characteristic syndrome of episodic stupor has been induced by the oral administration of substances which contain or liberate ammonia.

2. The onset of symptoms has been correlated with a sharp rise in the levels of ammonia in the peripheral blood and with reversible electroencephalographic changes similar in many respects to those observed in "hepatic coma."

3. The possible implications of these observations in providing an explanation of the "meat intoxication" syndrome in dogs with Eck fistula and of "hepatic coma" in man have been discussed.

Addendum: The patient died on July 30, 1953, after this article was submitted for publication. Death did not appear to be related to hepatic insufficiency or ammonia intoxication but rather to age, arteriosclerotic heart disease and possibly nutritional deficiency. At autopsy the liver showed diffuse fatty infiltration but no cirrhosis. There was degeneration of the cerebral cortex in places and throughout the gray matter a diffuse hyperplasia of the protoplasmic astrocytes. No evidence of carcinoma of the pancreas was found.

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STUDIES MADE BY SIMULATING SYSTOLE AT NECROPSY. III. ON THE GENESIS OF THE SYSTOLIC WAVES OF THE BALLISTOCARDIOGRAM [1]

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(Submitted for publication June 19, 1953; accepted September 18, 1953)

In a previous communication experiments have been described (1) in which, when systole was simulated in cadavers, the size of the ballistocardiogram proved to be dependent on two major factors: First, on the energy of the systolic blow; and second, on a factor which may be thought of as determining the hardness or softness of the blow, a factor varied in our experiments by changing the padding on the mallet whose strokes provided the energy for the simulation of systole. In this paper we seek to unite these two major factors in the genesis of the ballistocardiogram into a single conception and to ascertain its relation to more familiar aspects of cardiac function. Put another way we have asked ouselves the question whether there is a single aspect of cardiac performance which is driving the ballistocardiogram and if so, how changes in that aspect are related to changes in the size of this record.

In making this study we shall take full advantage of the curves which record both cardiac output and blood pressure at each instant, which are the chief features of our experimental preparation, and which permit us to estimate many aspects of "cardiac" performance with an accuracy not as yet approached in measurements made on living men or animals. In this paper we will present our data in detail. The results show that there is indeed a single aspect of cardiac function, related to the heart's force, so closely correlated with the amplitude of the ballistocardiogram under such a great variety of conditions that the magnitude of the one can properly be thought of as determining that of the other.

EXPERIMENTS

The apparatus is shown in Figure 1. It and the conduct of the experiments have been described in detail (1, 2). The results to be presented here were secured by simulating systole in cadavers lying on a ballistocardiograph. All the experiments but one were performed between 3 and 12 hours after death; one, through an error, was performed 24 hours after without any noteworthy difference in result. Normal or abnormal diastolic pressure levels were secured by an infusion of blood into the femoral artery, which was terminated just before systole. Systole was simulated by driving blood into the aorta and pulmonary artery by a blow from a heavily padded mallet falling through a known arc, which forced in the pistons of the two syringes.

Both peripheral and central blood pressures were measured continuously by Lilly capacitance manometers (3) while "cardiac output" at each instant of systole was registered by a record of the position of the syringe pistons made by a slit and light beam system.

Occasionally this technique was varied. In systoles No. 10 and 11 on J. W., to get widely diverse cardiac ejection curves, the syringes were pushed in by hand instead of by a blow from the mallet. In systoles No. 2 and 3 in M. M., blood pressure was measured in the pulmonary artery instead of the aorta. Figure 2 gives typical examples of the types of records secured.

In the conduct of the experiments we sought great diversity. Thus the stroke volume varied from 69 to 23 cc., the duration of ejection corresponded to that of pulse rates ranging from 200 to 30 per minute, the blood pressure varied from a systolic of 198 and a diastolic of 114 mm. Hg to a systolic of 52 and a diastolic of 29; some subjects showed advanced arteriosclerosis while in others the vessels were normal. We were also at pains to produce great variation in the contour of the cardiac ejection curves.

MEASUREMENTS AND CALCULATIONS

Blood pressure, pulse wave velocity, duration of ejection, and ballistocardiograms were estimated from measurements made from the photographs with a millimeter rule and a right angle placed directly on the records; the slopes of the syringe ejection curves were measured by an angle protractor as is shown in Figure 3. Every measurement was checked by remeasurement long after the result of the first had been forgotten, and we finally convinced ouselves that making the readings under magnification did not improve our accuracy.

The two authors checked the accuracy of their measurements against each other. In measuring the slopes

[1] This work was supported by a research grant from the National Heart Institute of the National Institutes of Health, United States Public Health Service.

[2] Markle Scholar in Medicine.

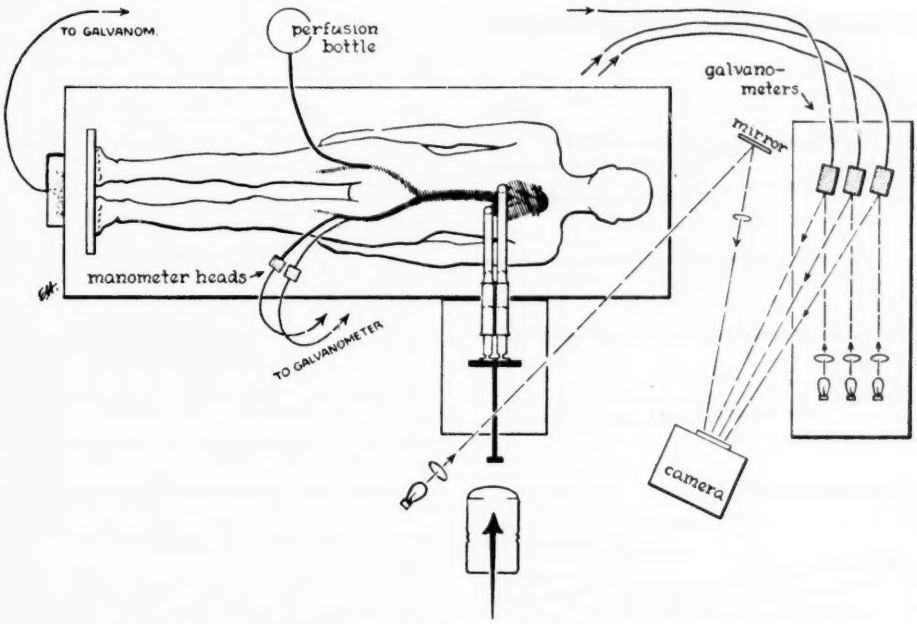

FIG. 1. GENERAL ARRANGEMENT FOR SIMULATING SYSTOLE IN A CADAVER LYING ON A BALLISTOCARDIOGRAPH

The details of the apparatus have been described (1, 2) and a drawing showing the apparatus as viewed from the side has also been published (1).

In brief: The suitably curved glass cannulas shown are of large diameter and their tips are tied into the mouths of the aorta and pulmonary artery. Glass tubes of similar size connect these cannulas with the syringes. The heavily padded mallet is suspended from an axis by a long handle which, at right angles to the plane of the figure, cannot be shown in it. Systole is simulated by letting this mallet fall through a known arc and its blow drives in both the syringe pistons and the slit which records their position: however, blows strong enough to completely empty the syringes are never used so the amount of blood injected varies with the conditions of each experiment. Blood pressure is recorded in two places (usually in the arch of the aorta and femoral artery as shown, sometimes in the pulmonary artery) by two Lilly manometers attached to minute plastic tubes inserted into the vessel through needles and advanced until their tips were in the desired position. The manometers and the ballistocardiograph are connected with mirror galvanometers and records are made by light beam systems and a photokymograph. The perfusion bottle attached to a cannula in the femoral artery is elevated at different distances above the cadaver and so perfusion from it provides any desired "diastolic" pressure before each "systole" is delivered.

of the steepest part of our cardiac ejection curves, where it was important to our thesis to get as high accuracy as possible, the authors reading the angles independently seldom differed by more than 1 and never by more than 2°. When measuring distances used in estimating blood pressure, pulse wave velocity, wave durations etc., they usually agreed within 0.3 mm., a distance which corresponded to about 1 mm. Hg pressure and 0.006 sec. time.

We were content to read the ballistocardiogram wave amplitudes to the nearest millimeter and the readings were corrected to a standard displacement of 1 cm. for 280 grams force. The decimal points which appear on the tables are due to this correction.

The total stroke volume was measured both from the change in the record and by reading the scale on the syringes before and after each systole. These values seldom differed by more than 2 cc. Duplicate readings of the syringe scale often differed by 1 cc.

For purposes of reproduction in one place the curves of cardiac output at each instant of systole were magnified 2.5 times and transposed to coordinate paper by a pantograph. These transposed curves were brought together on a single sheet by tracing them over a strong light. After inking the curves with the aid of a flexible ruler, they have been reproduced as Figure 4. However, all measurements of the slopes of the curves were made from the original records, rather than from the enlargements, which suffered some distortion in the process.

The statistical analysis of the data was performed with

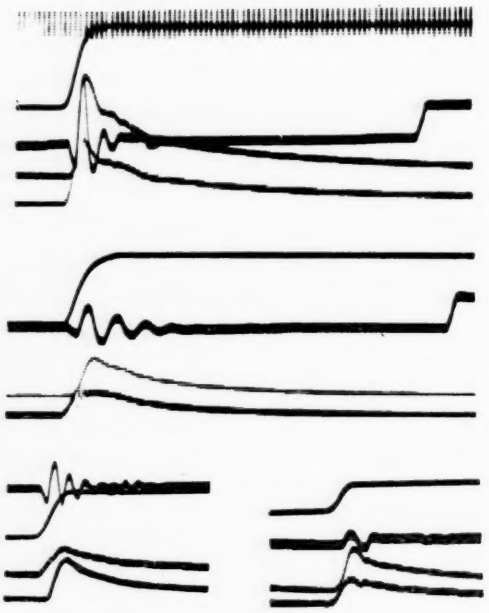

FIG. 2. FROM TYPICAL RECORDS OF SIMULATED SYSTOLES IN CADAVER PREPARATIONS

Top. Systole No. 6 on cadaver H. Z. Time is at the top, the shortest interval 0.04 sec. The record next below is that of the movement of the syringe pistons and so of the cardiac output. Next record below is the ballistocardiogram, the systolic deflections are shown towards the left; its calibration, the deflection carried by the removal of a force of 280 grams, is at the extreme right. The next record is femoral blood pressure, the bottom record aortic blood pressure; their calibrations are not reproduced.

Middle. Systole No. 1 on M. L. The film speed is identical with that of the top record. The cardiac output record and the ballistocardiogram, which could be readily distinguished in the original photograph, are superimposed at the left; again the ballisto calibration is seen at the extreme right. The femoral blood pressure record is above the aortic.

Bottom left. Systole No. 8 on J. W. Time is not shown and film speed was similar to the top record. The ballistocardiogram is above the cardiac output record. The aortic pressure record is above the femoral.

Bottom right. Systole No. 13 on H. Z. Time as in the top record. The records are in the same order, from above downward, as in the top record.

In all records the correction required for alignment is negligible. The reproductions are ⅝ actual size.

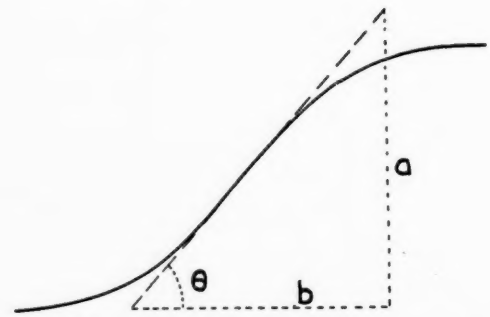

FIG. 3. METHOD OF MEASURING MAXIMUM SLOPE OF CARDIAC OUTPUT CURVES

The angle protractor straight edge was placed to coincide with the maximum slope and the angle with the horizontal, θ, was measured. Its tangent, the ratio a/b, was obtained from standard tables. The maximum velocity in cc. per sec. was secured by applying the calibration of the cardiac output record to the vertical component, *a*, and that of the time record to the horizontal component, *b*.

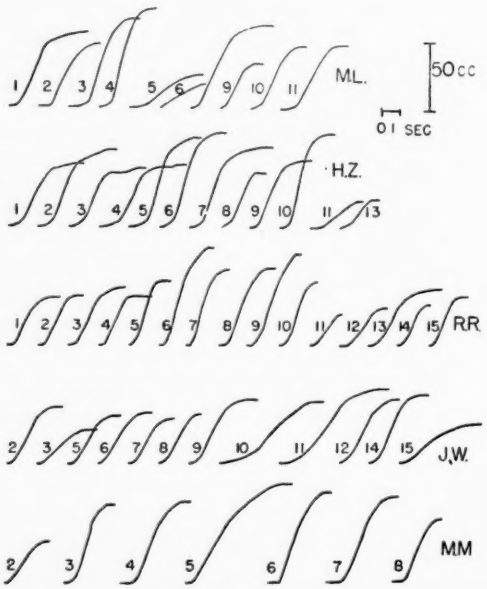

FIG. 4. CURVES OF CARDIAC EJECTION DURING SYSTOLE

The numbers correspond to the systoles as numbered in the tables and the curves of each subject have been placed in a row and can be identified by the initials placed after the last curves. The calibrations for cardiac output and for time are in the upper right corner, and apply to all the curves. The reproductions are ⅝ the size of the original curves.

TABLE I

Data from experiments on M. L.

Curve no.	Systolic blow	Padding number	Aortic B.P.		Femoral B.P.		Stroke volume	Duration of eject.	Pulse wave velocity	Ballistocardiograms					
										I Wave		J Wave		K Wave	
			Syst.	Dias.	Syst.	Dias.				Size	Dur.	Size	Dur.	Size	Dur.
	ergs $\times 10^{-6}$		*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*cc.*	*sec.*	*m. per sec.*	*mm.*	*sec.*	*mm.*	*sec.*	*mm.*	*sec.*
1	14.8	2	104	60	104	55	61	0.45	5.1	2.4	0.09	5.6	0.12	4.8	0.11
2	14.8	2	89	52	94	47	54	0.36	5.1	1.7	0.08	7.8	0.11	6.1	0.10
3	32.8	2	107	57	118	57	63	0.28	4.6	3.3	0.08	9.2	0.11	7.5	0.12
4	32.8	2	110	57	118	57	69	0.24	4.9	4.4	0.08	11.6	0.10	10.7	0.13
5	5.2	2	81	67	86	69	24	0.46	5.0	0.9	0.08	2.7	0.10	2.7	0.12
6	5.2	2	67	54	75	60	18	0.32	4.1	0		2.6	0.10	0.9	0.10
8	14.8	2	67	27	70	29	62	0.49	3.8	2.5	0.08	7.5	0.13	5.0	0.11
9	14.8	2	165	116	172	114	38	0.30	8.8	0.8	0.08	5.1	0.12	3.4	0.09
10	14.8	2	110	76	121	79	46	0.32	4.6						
11	14.8	2	100	72	109	69	48	0.35	5.6						

the aid of a Marchant calculator, using standard methods (4).

MATERIAL

The main characteristics of these subjects should be presented here, though they have been given in more detail before (1). M. L., a woman of 43, weight 70 kilos, died of leukemia; there was a mild degree of atheroma of the great vessels. H. Z., a man of 67, weight 74 kilos, died of cardiac infarction, and had moderate arteriosclerosis. R. R., a woman of 74, weighed 49.5 kilos and died of carcinoma of the colon; there was marked widespread arteriosclerosis. J. W., a woman of 68, weight 43 kilos, died of carcinoma of the sigmoid; there was widespread arteriosclerosis. M. M., a man of 43, weight 98 kilos, died of myelogenous leukemia; the arteries were judged to be normal. None of these subjects had hypertension during life.

RESULTS

In Tables I to V inclusive are the results secured in 54 consecutive systoles performed on the five cadavers perfused with blood. The numbers given to these systoles in the tables are not always consecutive because various calibrations were also given numbers, and the record of an occasional systole was lost through failure of the camera shutter to open. No data obtained on any systole on which a record was secured have been omitted.

The data given in the tables need explanation on only a few points. The padding number increases as more padding was placed on the mallet, and its exact meaning has been described (1). The vertical amplitudes of the ballistocardiogram

TABLE II

Data from experiments on H. Z.

Curve no.	Systolic blow	Padding number	Aortic B.P.		Femoral B.P.		Stroke volume	Duration of eject.	Pulse wave velocity	Ballistocardiograms					
										I Wave		J Wave		K Wave	
			Syst.	Dias.	Syst.	Dias.				Size	Dur.	Size	Dur.	Size	Dur.
	ergs $\times 10^{-6}$		*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*cc.*	*sec.*	*m. per sec.*	*mm.*	*sec.*	*mm.*	*sec.*	*mm.*	*sec.*
1	14.8	2	134	68	133	64	48	0.36	8.9	3.8	0.062	9.2	0.10	2.3	0.09
2	14.8	2	139	63	139	59	56	0.36	10.6	4.2	0.064	12.5	0.09	4.2	0.08
3	14.8	3	141	76	150	72	44	0.25	11.1	2.9	0.064	9.8	0.086	3.3	0.08
4	14.8	3	128	62	133	63	47	0.34	11.1	2.6	0.064	8.7	0.10	3.5	0.08
5	32.8	3	196	76	193	78	67	0.35	10.6	4.2	0.06	15.0	0.096	5.4	0.08
6	32.8	3	168	67	190	69	69	0.34	11.1	5.6	0.064	16.1	0.088	7.3	0.08
7	14.8	2	78	36	88	40	63	0.42	6.1	7.7	0.082	18.5	0.082	14.6	0.072
8	14.8	2	168	90	173	89	42	0.20	11.5	4.2	0.06	13.4	0.09	8.4	0.08
9	14.8	2	123	62	138	65	51	0.32	11.1	2.2	0.05	10.4	0.10	4.4	0.10
10	32.8	2	193	71	198	74	68	0.26	10.6	3.1	0.04	16.1	0.10	0	
11	5.2	2	91	64	95	70	20	0.24	10	0		1.5	0.12	0	
13	7.8	2	128	89	138	93	20	0.18	12.8	0		3.4	0.12	0.9	0.06

TABLE III

Data from experiments on R. R.

Curve no.	Systolic blow	Padding number	Aortic B.P.		Femoral B.P.		Stroke volume	Duration of eject.	Pulse wave velocity	Ballistocardiograms					
										I Wave		J Wave		K Wave	
			Syst.	Dias.	Syst.	Dias.				Size	Dur.	Size	Dur.	Size	Dur.
	ergs $\times 10^{-5}$		*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*c.c.*	*sec.*	*m. per sec.*	*mm.*	*sec.*	*mm.*	*sec.*	*mm.*	*sec.*
1	14.8	2	114	72	125	74	36	0.29	7.0	5.1	0.07	7.3	0.10	5.1	0.08
2	14.8	2	95	54	109	85	38	0.28	5.7	6.1	0.09	12.5	0.09	11.0	0.07
3	14.8	2	68	38	73	36	43	0.32	5.3	4.6	0.09	9.1	0.11	4.6	0.06
4	14.8	2	116	68	121	66	36	0.26	6.2	5.6	0.08	10.6	0.09	6.7	0.07
5	32.8	2	146	72	155	69	46	0.22	9.1	13	0.08	25.4	0.08	21.6	0.06
6	32.8	2	89	27	62	16	69	0.30	4.2	14.9	0.09	35.8	0.10	26.8	0.06
7	32.8	2	149	64	127	43	54	0.26	7.0	12.2	0.08	23.9	0.08	23.3	0.06
8	32.8	3	145	61	145	66	58	0.31	6.0	4.9	0.08	11.9	0.10	8.1	0.06
9	32.8	3	65	31	69	30	69	0.30	3.3	17.5	0.13	15.5	0.09	10.5	0.07
10	32.8	3	169	77	174	78	50	0.26	7.0	5.8	0.08	14.2	0.08	11.6	0.06
11	11.7	3	114	81	116	79	23	0.30	7.0	1.6	0.07	3.7	0.09	2.1	0.10
12	11.7	3	95	64	103	65	29	0.27	6.6	1.7	0.10	4.5	0.09	1.7	0.07
13	11.7	3	54	34	52	33	41	0.45	3.8	3.3	0.09	6.1	0.11	1.7	0.06
14	14.8	2	111	65	110	64	30	0.20	7.0	3.3	0.08	7.2	0.10	6.7	0.07
15	14.8	2	119	75	115	65	36	0.16	7.5	5.9	0.07	12.3	0.10	5.0	0.06

waves were measured from the records' base line.

The systolic ejection curves given in Figure 4 are numbered to correspond with the curve number in the tables, as are the points in Figures 5, 6 and 7.

ANALYSIS OF RESULTS

We will present our analysis of the results in much the same order as our ideas developed.

We started this study by plotting the sum of the vertical depth of the I wave and the vertical height of the J wave, to be called the I + J distance, against the cardiac output as the results of each experiment came in; typical plots for four subjects are given in Figure 5. Inspection of these figures show at once that the correlation is remarkably good for the M. L. data, where the regression is evidently curved, but the scatter is greater in the data secured on H. Z. and R. R., and is much greater for that of M. M. Inspection of the data secured on the last three subjects shows that the greater scatter is largely due to results obtained in systoles in which the mallet padding was increased (results represented by underlined dots in Figure 5), these values tending to gather to the right and below the other data. Indeed, in

TABLE IV

Data from experiments on J. W.

Curve no.	Systolic blow	Padding number	Aortic B.P.		Femoral B.P.		Stroke volume	Duration of eject.	Pulse wave velocity	Ballistocardiograms					
										I Wave		J Wave		K Wave	
			Syst.	Dias.	Syst.	Dias.				Size	Dur.	Size	Dur.	Size	Dur.
	ergs $\times 10^{-5}$		*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*c.c.*	*sec.*	*m. per sec.*	*mm.*	*sec.*	*mm.*	*sec.*	*mm.*	*sec.*
2	15.2	2			102	50	43	0.32		3.3	0.06	3.7	0.13	0	0
3	11.0	2			81	53	26	0.33		2.5	0.06	2.5	0.09	2.8	0.06
5	15.2	2			90	48	36	0.34		3.3	0.06	5.4	0.10	0	
6	15.2	2	94	52	96	51	37	0.33	5.8	3.3	0.07	7.5	0.07	2.0	0.04
7	15.2	2	115	73	111	67	32	0.25	8.2	5.0	0.07	5.8	0.08	2.5	0.05
8	15.2	2	102	60	96	52	36	0.26	6.6	3.3	0.06	7.5	0.08	2.0	0.04
9	15.2	2	56	29	67	31	48	0.39	5.1	4.2	0.06	5.8	0.09	2.0	0.04
10	Hand	2	105	77	89	59	46	0.58	5.1	0.4	0.04	3.3	0.09	2.6	0.05
11	Hand	2	88	48	86	46	55	0.65	5.1	0	0	4.6	0.08	2.5	0.05
12	14.2	2	82	45	89	48	48	0.36	6.0	3.3	0.06	5.0	0.12	1.5	0.03
14	17.3	2	117	71	105	43	51	0.35	7.3	5.0	0.07	7.5	0.10	2.5	0.05
15	17.3	2	42	29	42	29	30	0.44	5.1	1.7	0.07	2.5	0.13	2.0	0.04

TABLE V

Data from experiments on M. M.

Curve no.	Systolic blow	Padding number	Pul. art. or aortic B.P.		Femoral B.P.		Stroke volume	Duration of eject.	Pulse wave velocity	Ballistocardiograms					
										I Wave		J Wave		K Wave	
			Syst.	Dias.	Syst.	Dias.				Size	Dur.	Size	Dur.	Size	Dur.
	ergs $\times 10^{-6}$		*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*cc.*	*sec.*	*m. per sec.*	*mm.*	*sec.*	*mm.*	*sec.*	*mm.*	*sec.*
2	7.8	2	46	1	94	63	28	0.28		0	0	2.8	0.13	0.4	0.14
3	31.1	2	41	8	94	49	54	0.25		2.0	0.06	11.3	0.10	14.1	0.11
4	19.2	2	68	49	80	51	58	0.36	4.8	1.6	0.05	7.3	0.11	8.1	0.09
5	19.2	4	77	61	80	61	68	0.64	4.6	0.4	0.08	1.6	0.11	1.6	0.28
6	19.2	1	81	66	83	66	62	0.32	5.0	4.8	0.08	11.3	0.08	8.9	0.08
7	19.2	2	52	38	59	36	60	0.38	3.4	1.6	0.05	4.8	0.10	6.4	0.10
8	19.2	2	103	80	115	84	44	0.28	5.6	2.9	0.06	5.6	0.09	5.6	0.09

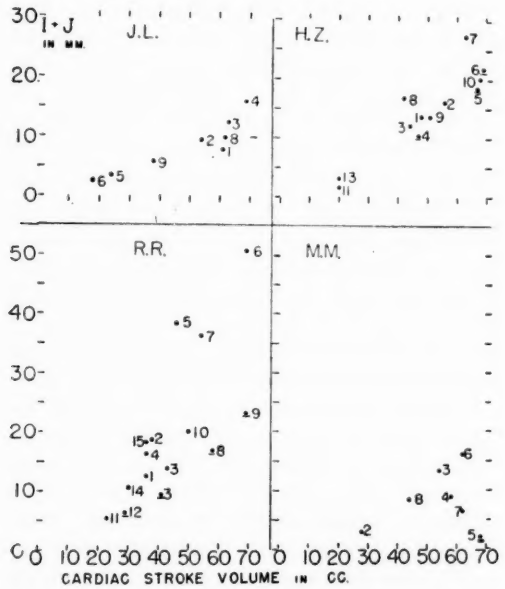

FIG. 5. DOT DIAGRAMS SHOWING THE RELATION OF THE BALLISTIC AMPLITUDE, AS MEASURED BY THE VERTICAL I + J DISTANCE, TO THE SYSTOLIC STROKE VOLUME IN EXPERIMENTS ON FOUR SUBJECTS

The data on any subject are to be identified by the initials. The numbers adjacent to the points correspond to the numbers given systoles in the tables and in the curves in Figure 4. In diagram at upper left J.L. should be M.L.

The results of certain experiments in which extra padding was added to the mallet can be identified by bars under the dots.

the data of R. R. inspection suggests that there is one regression for the harder and another for the softer blows. Obviously, the reason for the better agreement in the case of M. L. is that the same mallet padding was used throughout this experiment. One therefore must conclude that the size of the I + J distance would measure the cardiac output well, only in the absence of such changes in cardiac function as are simulated by increasing the mallet's padding. That variations in cardiac function causing a change in the form of the cardiac ejection curve would alter the relation between the amplitude of the record and the cardiac output was realized in our first communication on this subject (5), and a further study of the important relationship between the ballistocardiogram and cardiac output will be the subject of another paper.

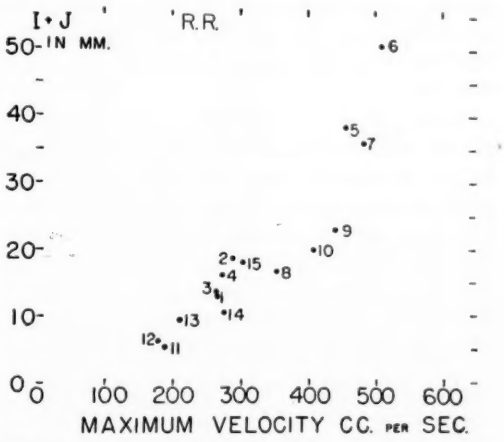

FIG. 6. DOT DIAGRAM SHOWING THE RELATION OF BALLISTIC AMPLITUDE TO THE MAXIMUM VELOCITY OF CARDIAC EJECTION

The numbers next to the dots correspond with the numbers of systoles in Table III. Data from subject R. R.

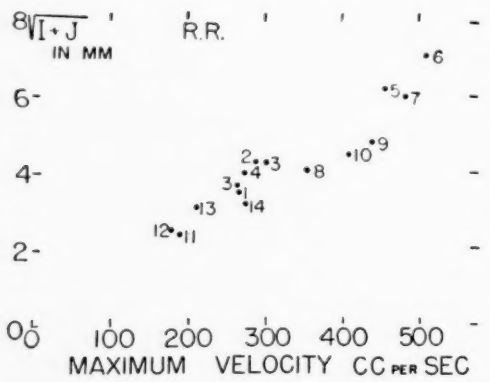

FIG. 7. DOT DIAGRAM SHOWING RELATION OF THE SQUARE ROOT OF BALLISTIC AMPLITUDE TO THE MAXIMUM VELOCITY OF CARDIAC EJECTION

The numbers are as before. Data from subject R. R.

Comparing the vertical J + K distance with cardiac output leads to still greater scatter and we have not reproduced the dot diagrams. Obviously the size of the cardiac output, considered by itself, is not the prime factor in determining the ballistocardiogram's amplitude.

As the data on M. L. appear to conform to a curve convex upward the next step is obviously to try the effect of plotting the square root of the I + J values against the stroke volume. This change diminishes the scatter in some subjects and the regression more nearly passes through the zero point, but it does not meet the need of pulling into line the results secured after the softer systolic blows.

Plotting the I + J amplitude, or $\sqrt{I + J}$, against the left ventricular work or power, both calculated for us dynamically from the stroke volume and aortic pressure curves by Mr. Walter Feder, likewise does not lead to improvement in the scatter, and the value secured after the systoles made with heavier padding are still outlying to the right and downward. The relation of our results to such estimates of work or power will be studied further so these dot diagrams will not be reproduced here.

The plan of comparing the ballistic amplitude directly with the more familiar aspects of cardiac function not having yielded a close correlation, we turned to analysis of the cardiac output curves given in Figure 4 and soon had a clue. When the force of the blow is diminished by dropping the mallet through a smaller arc both the stroke volume and the slope of the cardiac ejection curve are diminished; the curves given in Figure 4 show many examples of this. In a few instances, however, pairs of experiments can be found in which the cardiac output is the same in each, but the slope of the ejection curve differs. Thus if one looks carefully at the curves No. 7 and 8 on R. R. in Figure 4, and then inspects the corresponding dots in Figure 5, one sees that the cardiac output is nearly the same in both, but the ejection curve of No. 7 rises faster than that of No. 8. The same can be said of curves 9 and 6 of R. R. and for other pairs of curves in other subjects, although the difference between the slopes is seen with much more difficulty in the reduced Figure 4 than in the original records. In these pairs the steeper curve is always accompanied by a ballistocardiogram of greater amplitude, so we are led to inspect the relation between the square root of the amplitude of the I + J waves and the slope of the ejection curve at the steepest point. A plot of I + J, or $\sqrt{I + J}$, against the maximum slope of the curve measured as the angle from the horizontal in degrees showed too much scatter to be promising, but it was soon apparent that what was needed was not the angle itself but its tangent, which measures the maximum velocity attained during the ejection.

In Figure 6 the slopes of the curves of R. R., estimated from the tangent of the angle made by the steepest part of the curve with the horizontal, corrected by the calibration of the vertical limb in cc. and the horizontal limb for time, are plotted against the I + J distance. Comparison with Figure 5 shows that the improvement in scatter is remarkable. The regression is now plainly curved by using $\sqrt{I + J}$ instead of I + J and we get the much straighter alignment shown in Figure 7. Obviously, we are now on a promising line of attack.

The plot of $\sqrt{I + J}$ against maximal velocity for all five subjects is shown in Figure 8. Inspection shows that the correlation now is of a high order, $r = 0.82$, and one is certainly tempted to stop here. Nevertheless, further inspection shows that the data of the heaviest subject, M. M., tends to outlie to the right and downward, while that of the lightest subject, J. W., tends to outlie to the left and upward, a finding which plainly suggests

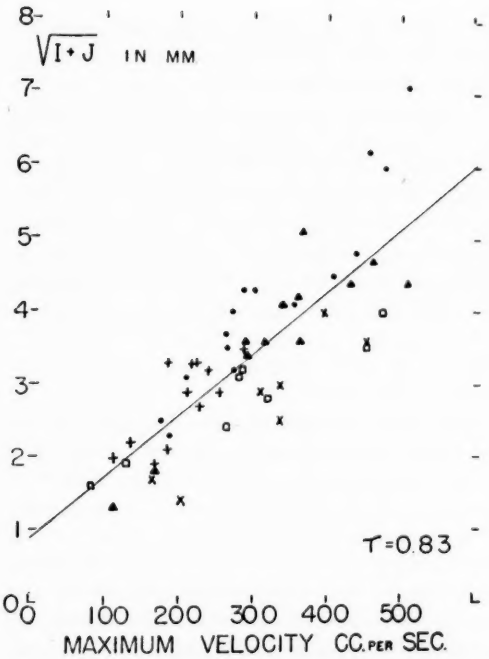

FIG. 8. DOT DIAGRAMS SHOWING THE RELATION BETWEEN $\sqrt{I+J}$ AND MAXIMUM VELOCITY OF EJECTION FOR ALL FIVE SUBJECTS

Dots = R. R.; triangles = H. Z.; squares = M. L.; crosses = J. W.; x's = M. M. The line is the calculated regression x on y.

that a consideration of the subject's size would further diminish the scatter.

So our next step was to plot the $\sqrt{I+J}$ against the maximal velocity of the ejection curve divided by the surface area for each subject, with the result shown in Figure 9; the correlation is now surprisingly good, $r = 0.92$ a value which, when tested by Fisher's Z function (4), proves to be significantly better than the correlation secured without considering the subject's size.

We studied the data further by measuring the time from the beginning of ejection to the point at which maximum velocity was reached. In most curves it was difficult to determine this point exactly and the two authors reading independently often differed considerably in their measurements. We made a dot diagram to compare the $\sqrt{I+J}$ with *maximum velocity ÷ the time required to attain it,* the quotient being average acceleration. The correlation was very evident but inspection showed that it was clearly not so good as that shown in Figures 8 and 9. The error in measuring the time required to attain maximum velocity was undoubtedly a large factor in the scatter and so we were discouraged from pursuing the matter further.

DISCUSSION

It seems proper to expect that such aspects of cardiac function as output, work, energy, force, and power will vary together throughout a wide range of physiological and clinical conditions, so it should occasion no surprise if the ballistic amplitude was found to be significantly correlated with more than one of them. But the wide range of our experimental conditions permits distinctions which would be difficult or impossible to draw from data secured during life.

The maximum velocity of our ejection curves is so closely correlated to the amplitude of the

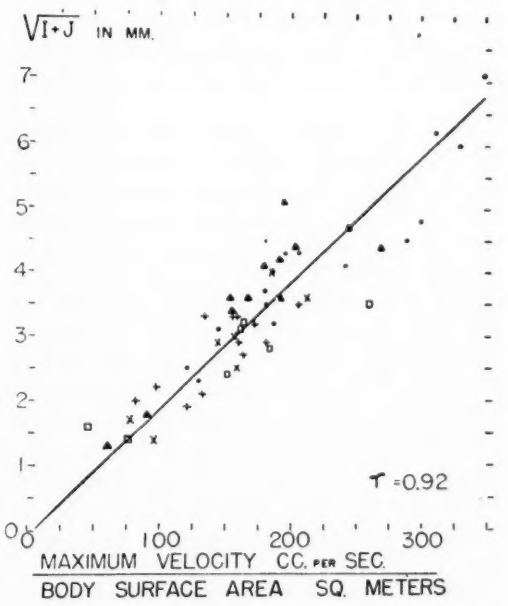

FIG. 9. DOT DIAGRAM SHOWING THE RELATION BETWEEN $\sqrt{I+J}$ AND MAXIMUM VELOCITY OF EJECTION DIVIDED BY BODY SURFACE AREA

Symbols as in Figure 8. The best line is the calculated regression x on y, this regression being used because we propose to employ its equation in estimating maximum velocity of ejection from $\sqrt{I+J}$, that is from the amplitude of the ballistocardiogram in any record.

ballistocardiogram that the known errors of reading the record may in large measure account for the scatter we find, and this relation holds so well throughout such a great diversity of experimental conditions that one has the right to expect it will hold for most of the common clinical conditions.

We believe that the maximum velocity shown by our curves gives us an indication of the force of the heart beat for the following reasons. By definition this force is estimated from the product of mass times acceleration, and the maximum velocity of our curves is the integral of the accelerations of which it is the result.

The maximum velocity recorded by our curves is in units of cubic centimeters per second; since the specific gravity of blood is close to one, the values for grams per second would be very similar. By consideration of the size of the aortic cannula or of the aorta itself, linear velocity could be readily computed, but when linear velocity varies with changes in the diameter of the conducting tubes the mass of the contained blood varies inversely, so the product of mass × veolcity, or of mass × acceleration, is independent of the vessel diameter (6).

Thus the amplitude of the ballistocardiogram seems to be plainly related to cardiac force, but we are not yet in position to estimate cardiac force in absolute units because of the uncertainty about the mass of the blood caused to move in the body by the heart's energy at any instant of time, a mass that certainly far exceeds the stroke volume itself because blood is pushed on ahead of that ejected by the heart. This difficulty has been discussed (2), and we propose to content ourselves with relative estimations of cardiac force until our ideas of the energetics of cardiac action are clearer then they are at present, and because of increasing doubts that the classic methods of estimating cardiac work provide the best approach to the problem.

The arrangement of our data reported in the preceding paper (1) showed that the amplitude of the ballistocardiogram is related to the total energy delivered to the circulation during systole only if that energy is supplied in a constant manner. Energy delivered rapidly produced a far larger ballistocardiogram than the same energy delivered slowly, so it appeared to be energy per unit of time which was the genesis of the ballistic deflections (1). The relationship of this conception to the data described in this paper is easy to see. In our experiments, when provided with little padding the falling mallet was stopped rapidly as it struck the syringe pistons, and the blood was accelerated rapidly by the energy thus rapidly delivered; when provided with much padding the falling mallet was stopped much more slowly and the blood was accelerated more slowly. So the relationship between our results and the force delivered to the circulation is plain. The interpretation described in this paper does much to clarify the ideas expressed more crudely before.

This new evidence that the amplitude of the ballistocardiogram is a measure of cardiac force comes as no surprise to us. The equation for force according to Newton's Laws was the basic equation from which our original treatment of the mathematical theory was derived (5); also, in our first experiments on the cadaver the data led to a similar conclusion; indeed, although the former method of measuring acceleration from our curves (2) was cruder than that described in this publication, the correlation coefficients relating ballistic amplitude to acceleration are almost identical in the two groups of data (2), and the improvement in the correlation in our recent work is manifest only by the fact that, dealing with larger numbers of estimates in this paper, the level required for significance is lower.

In our first study (2), the data did not permit a decision whether $I + J$ or $\sqrt{I + J}$ permitted a better estimate of cardiac performance. The newer data permit this decision; $\sqrt{I + J}$ is significantly better. Interestingly enough this was predicted from theory (5). We have also asked ourselves the question whether data based on the areas of the I and J waves, treated as triangles with their bases on the record's base line, would permit a better estimation of cardiac force than using the sum of the vertical depth of I and height of J. The equations given in Table VI show that the scatter about the two regressions is almost identical, but our early experience with dynamic calibration of ballistocardiographs (5) provides a strong reason for basing estimates on areas, because secondary vibrations may greatly affect the height of a wave's

TABLE VI

*Regression equations—maximum velocity as a relative measure of cardiac force as derived from measurements of the ballistocardiogram and the body surface area**

Equation no.		σ_{xy}
1	Maximum velocity *cc. per sec.* = 75.8 $\sqrt{\text{I} + \text{J amplitude } mm.}$ + 37	55.5 *cc. per sec.*
2	Max. vel. *cc. per sec.* = [body surface *sq. m.*][51 $\sqrt{\text{I} + \text{J amplitude } mm.}$ + 5]	28 *cc. per sec.*
3	Max. vel. *cc. per sec.* = [body surface *sq. m.*][252 $\sqrt{\text{I} + \text{J areas } mm.\ sec.}$ − 5]	29 *cc. per sec.*

* Vertical amplitudes of the ballistic waves measured in millimeters from the base line may be used in these equations only if the calibration of the ballistocardiogram is standard, *i.e.*, a force of 280 grams produces a deflection of 10 mm. on the record. If the calibration is not standard the measurements on the records must be corrected to this value before inserting them in these equations.

tip when they have little or no effect on the area of the wave.

While the clear relation of the ballistic amplitude to cardiac force indicated in our newer results was in accord with our expectations (1, 2), the advantage of multiplying the measurements made on the record by a factor related to body size came to us as a surprise. In our previous study (2) the three subjects whose data provided the comparison between acceleration and ballistic size had been of almost identical height and two were of identical weight. So the effect of size on the relationship was not apparent on inspection of the dot diagrams and the effect of the introduction of a size factor was not investigated.

Other aspects of our recent work have given indications of impedance in our system, and it seems proper to locate this in the body rather than in the apparatus. Our ballistocardiograph is so sensitive and so little damped that, in the absence of a body upon it, the vibrations in the building keep it in motion continuously, so it seems evident that no noteworthy impedance occurs in it. Place a body upon it and the light spot comes to rest, as can be seen by the straight base lines of our records. As the calibrations shown in Figure 2 indicate, the damping in the body is often close to critical when forces corresponding in magnitude to those which produce the ballistocardiograms are used to test it; but in certain other cadavers, similar tests indicate that the body's damping may also be somewhat less than critical. Obviously we have every right to locate the impedance in the body, and this provides a satisfactory explanation of other findings.

Thus, in the usual systole illustrated by the great majority of curves shown in Figure 4, the first ballistic deflection, the onset of the downstroke of the I wave, follows the beginning of ejection by an interval so short that it is hardly measurable; this is illustrated by the first three of the curves shown in Figure 2. But if the injection is initiated more slowly as in curves 10 and 11 in J. W. and in curve 13 in H. Z. in Figure 4, no deflection follows the onset of ejection for a much longer time; and, as can be seen in the last curve in Figure 2, the first deflection may then be headward. Indeed, in early experiments in which water was used as perfusing fluid, we were able to inject amounts over 50 cc. into each of the great vessels, by pushing in the syringes slowly by hand, without causing any ballistic deflection whatsoever, although certainly some force was being generated. Obviously therefore, small amounts of force generated deep in the body are so impeded that they do not "get through" to produce a deflection of the ballistocardiogram; and when the force generated is of a certain small magnitude, that which tends to cause the I wave may be blocked while the larger forces causing the J wave break through to produce a small deflection as is seen in the last curve of Figure 2. Thus by an injection which accelerates slowly we can reproduce complexes lacking an I wave which are similar to those found so frequently in records taken in the clinic, and we have a satisfactory explanation for this common abnormality of contour.

That increased fat would impede the delivery of the heart's energy to the ballistocardiogram was suspected by Bouhuys (7), and looking backward over older data from this laboratory, one sees that we have had other hints of the need of introducing a factor related to body size into our

basic equations. Indeed there was such a factor, "A", an estimate of aortic cross section area from the surface area and age of the patient, in our original cardiac output equation (5). Looked upon from the vantage point of 15 years experience, we see that "A" not only supplied the need for a factor related to body size, but also, because it increased with age, it provided a compensation for the diminishing size of the ballistocardiogram as age advances (8), and both aspects served to reduce the scatter of the normal standards. When we omitted "A" from our equation, for mathematical reasons which still seem valid (6), the scatter of the results secured on normal subjects increased considerably.

This newly demonstrated influence of body size throws light on a puzzling finding discovered in the data of other clinics besides this one (9–11). It has always been obvious by inspection that, on the average, men give larger ballistocardiograms than women, and women larger than children; so the concept that the amplitude of the ballistocardiogram is related to body size seems obviously correct. Indeed, if data from healthy men and women are combined in a single group, significant positive correlation between amplitude of ballistocardiogram and body surface area is readily demonstrated. But it came as a surprise to discover that in groups consisting of healthy adult males only, or of healthy women only, the correlation between ballistic amplitude and body surface was no longer significant. The reason now seems obvious. It is proper to regard the force of the resting heart beat as correlated with body size, but this relation is in part masked by the increased impedance of the larger bodies, so that one needs a large number of cases and a wide spread of the data before the correlation between ballistic amplitude and body size can be conclusively demonstrated.

Our results also throw light on one of the chief uncertainties of the field which may be called the errors in transmission. Even if ballistocardiographs could be made mechanically perfect, one could not avoid the hazard involved in attempting to record forces generated in the center of the body by apparatus placed outside it (12). Various parts of the body must be thought of as masses of tissue elastically connected one with another, and completely firm attachment of the elastic body to the rigid ballistocardiograph is obviously impossible to secure (13). These factors introduce distortion into the record, and so, as we have always emphasized, the ballistocardiogram cannot be expected to give a completely true account of the forces generated deep in the body of which it is a manifestation (5, 12, 14). While some data on the movement between the body and the table has been secured (7), and this subject deserves further study, the errors inherent in the transmission of forces along the mechanical train within the body have been more difficult to evaluate. The problem is not whether there is distortion, which we freely concede is to be expected, but whether the distortion is sufficiently uniform from time to time and from subject to subject to permit one to avoid the error of interpreting as changes in cardiac function, changes in the ballistocardiogram due in reality to differences in the body tissues, or in the attachment of the subject to the ballistocardiograph.

Evidence available for some years has clearly suggested that as far as the I and J waves are concerned, any distortion introduced by the errors of transmission behaves in an orderly way. The extraordinary similarity of contour of ballistocardiograms secured in healthy young adults of great diversity of size and body build, recorded by ballistocardiographs completely different in form and construction, can hardly be interpreted otherwise. But such data, impressive though they are, do not give quantitative information on the point.

In the experiments described in this paper, the ballistocardiograms secured are subject to all the errors of transmission which occur in similar records obtained during life, for we are recording forces generated deep in the body, indeed, generated at the same places and in the same manner as occurs during life, by apparatus placed outside the body; and the attachment of our cadavers to the ballistic table, as they lay on it with their heels firmly against the footplate, is identical with the technique we use when taking records during life.

We interpret the demonstration that the size of the body affects our results as providing the first important information about the errors of transmission. But, after correction for body size has been made, the correlation between our rela-

tive estimate of cardiac force and the amplitude of the ballistic record becomes so good that it seems evident that the other irregularities in the transmission of the forces affect little, if at all, our ability to form a quantitative estimate of the relative strength of the heart's beating by measuring the amplitude of the ballistocardiogram; and, in our data, the error of the estimate compares well with that of other useful clinical measurements such as the estimation of the blood pressure (2).

SUMMARY

The results of experiments are reported in which cardiac systole was simulated at necropsy by simultaneous and similar injections of blood into the aorta and pulmonary artery, after a systemic diastolic pressure had been secured by an infusion of blood into the femoral artery. These experiments were conducted under widely different conditions of stroke volume and blood pressure, and both in arteriosclerotic subjects and in those with normal vessels.

The amplitude of the resulting ballistocardiograms, determined by the vertical distance between I and J wave tips, or by the sum of the areas of the I and J waves, correlated well with the "cardiac outputs" in certain types of experiments, but under other conditions the relation changed. Similarly the relation between the ballistic amplitude and "cardiac" work or power did not hold well throughout the varying conditions existing in the experiments.

However, a study of the curves of systolic ejection of blood at each instant of time indicated that the square root of the amplitude of the ballistocardiogram, $\sqrt{I + J}$, correlated well with the maximum velocity of ejection throughout the range of our experiments; and this relation was significantly bettered by the inclusion of the body surface area as a factor in the equation, so that the final correlation was most gratifying.

As the maximum velocity of the injected blood is equal to the integral of the acceleration which preceded it, it is concluded that the amplitude of the ballistocardiogram is related to the force of the heart, and that this relation holds well over a wide range of clinical conditions. However, the larger the subject's body, the greater the impedance to the delivery of the heart's force to the ballistocardiograph, so that a correction for body size improves the estimate of the heart's force from measurements made on the ballistocardiogram.

While uncertainties about the fundamental dynamics of cardiac action prevent an estimation of cardiac force in absolute units, it seems entirely proper to employ the ballistocardiogram to estimate cardiac force in relative terms, and thus compare the strength of the heart's contraction in normal and abnormal persons, and under many different physiological and clinical conditions.

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THE EFFECT OF HABITUALLY HIGH IRON INTAKE ON CERTAIN BLOOD VALUES IN PREGNANT BANTU WOMEN

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The influence of pregnancy on certain blood values (hemoglobin, hematocrit, serum iron, and total iron binding capacity) and their significance in the "anemia of pregnancy" have been widely investigated.

Combined evidence indicates that as pregnancy proceeds, a significant fall occurs in levels of hemoglobin and hematocrit, the explanation being that the increase in plasma volume exceeds the total increases in hemoglobin and red cells (1). In addition, evidence shows a significant rise to occur in total iron-binding capacity (2–4). There is not the same unanimity, however, with serum iron findings. Laurell (2), Lundström (5) in Sweden, and Ventura and Klopper (4) in England, have reported a fall in this value, detectable at mid-term. Yet, in America, neither Fay, Cartwright, and Wintrobe (6) nor Rath *et al.* (3) found a significant change in serum iron during pregnancy; the latter authors, therefore, alleged that the Swedish data indicated widespread iron deficiency in that country. Presumably, the same inference applies to the English series.

In South Africa, we have been greatly interested in the iron metabolism of the Bantu because: (*a*) their iron intake is often unusually high, sometimes as much as 200 mg. per diem, derived not only from the element present in their customary diet, but from adventitious iron taken up from iron utensils during the preparation of food (7); (*b*) abnormally high serum iron values are occasionally encountered in adults (8); this investigation we have confirmed and considerably extended (9); (*c*) hypochromic anemia is extremely rare (10); (*d*) at necropsy, abnormal deposition of iron is frequently seen in the tissues of the adult Bantu (11–13). We thought, therefore, that it would be illuminating to determine the above blood values, particularly serum iron levels, in groups of pregnant and non-pregnant Bantu women whose everyday diet, although deficient in many respects according to accepted standards, is characterized by an *habitually* high iron content.

MATERIALS AND METHODS

The Bantu women, examined while attending health clinics, were partly from the Johannesburg urban area, though mainly from two rural native settlements near Pretoria. Subjects were thus dwelling 4,000 to 5,000 feet above sea level. This fact must be taken into account, bearing in mind that hemoglobin concentration increases with altitude (14). Obviously, it would have been desirable to have followed a series of subjects throughout the period of gestation. But attendance at clinics is most irregular; moreover, many pregnant women do not attend until several months after conception.

The major source of calories for these people is maize, which is taken largely in the forms of cooked and sour porridges. This cereal is also used, with "kaffircorn" (sorghum), in the making of kaffir beer. These foodstuffs are usually prepared in the ubiquitous iron kaffir pots, though occasionally in paraffin cans. Several samples of foodstuffs were obtained from patients. The iron contents were determined by the thioglycollic acid method (15). Results are given in Table I.

Blood samples were taken by venipuncture between 9 a.m. and 10:30 a.m., the usual precautions being taken to prevent iron contamination. Determinations of hemoglobin, serum iron, and iron binding capacity were carried out in duplicate. Hemoglobin values were determined by the oxyhemoglobin method, using a Hilger-Spekker absorptiometer (Ilford filter 602), calibrated against total iron determinations. For hematocrit, Wintrobe tubes were used, centrifuging for one hour at 3,000 r.p.m. Serum iron was determined by the thioglycollic acid method, and iron binding capacity according to the method of Rath and Finch (16). The total iron binding capacity was calculated by adding the serum iron value to the iron binding capacity.

Since Rath *et al.* (3) have shown that the main increase in iron requirement during pregnancy occurs towards the end of the second trimester, and since the Swedish (2, 5) and English (4) workers have reported a fall in serum iron at about mid-term, we divided our subjects into two groups, one from 4 to 22 weeks, and the other from 26 to 40 weeks duration. Results are given in Table II.

TABLE I

Iron content of prepared foodstuffs consumed by our subjects

	Cooked porridge	Sour porridge	Kaffir beer
No. of samples	20	18	12
Iron content : mean range	9.9 mg./Fe/100 Gm. dry wt. (4.8–19.7)	45.0 mg./Fe/100 Gm. dry wt. (6–145)	4.2 mg./Fe/100 ml. (1.1–12.0)

N.B. South African maize contains 0.2 to 18.0, average 3.4 mg. Fe per 100 Gm. dry weight (17).

DISCUSSION

Dietary surveys by Smit (18) reveal that the quantity of maize consumed daily by the urban Bantu ranges from 6 to 21 oz. (170 to 590 Gm.) per head; that of wheat is from 5 to 15 oz. (140 to 420 Gm.). Information obtained by our dietitian, Miss J. L. Seward, on visiting homes of patients, together with information given by the patients themselves, suggest that: (*a*) the consumption of maize per diem by our subjects averaged about 1 lb. (450 Gm.); (*b*) about four-fifths of the maize bought is consumed as cooked porridge and the remainder as sour porridge; and (*c*) our subjects consumed an *average* of about 100 ml. kaffir beer per diem.

Using data given in Table I, the iron contribution by 0.8 lb. (360 Gm.) cooked maize ranged from 17 to 70 mg.; that from 0.2 lb. (90 Gm.) sour porridge, 5 to 120 mg.; that contained in 100 ml. kaffir beer, from 1.1 to 12.0 mg. Hence, the total iron ingested from these sources ranged from 23 to 202 mg., the mean value being 171 mg. per diem. Of course, additional amounts of iron are supplied by other components of the diet, *e.g.*, bread, legumes, vegetables and meat. This unusually high iron intake may be compared with the allowance currently recommended for pregnant women, namely, 15 mg. iron per diem (1)—a level not always reached by such subjects in the United States or Britain (19, 20). While we do not wish to attach undue importance to our figures, it must be stressed that these people are *habituated* to an iron intake *far higher* than that of Europeans, although the diet is deficient in several respects according to accepted standards.

Table II indicates that during pregnancy there is a significant rise in total iron binding capacity, but there is *no* significant fall in hemoglobin, hematocrit, nor serum iron.

As mentioned in the introduction, during pregnancy it is usual for hemoglobin concentration and hematocrit value to fall to a variable extent. These reductions, however, may be regarded as "physiological" and not essentially as indicative of iron deficiency. Hypochromic anemia from the latter cause is considered to be present only when the reduction in hemoglobin concentration reaches a certain level, normally placed at 11 Gm. per 100 ml. (21). The constancy of hemoglobin concentration and hematocrit levels during the pregnancy of the Bantu subjects is therefore most remarkable. One possibility is that there has been no increase in

TABLE II

Blood values in non-pregnant and pregnant Bantu women (18 to 40 years)

Period of pregnancy	Hemoglobin	Hematocrit	Serum iron (γ/100 ml. serum)	Total iron-binding capacity (γ/100 ml. serum)
	Gm. %	*%*		
Non-pregnant (48)*	13.9 ± 0.9 (12.3–16.0)	42.9 ± 2.9 (38–49)	119 ± 32 (65–195)	323 ± 45 (225–445)
0–22 weeks (43)*	13.9 ± 0.9 (12.2–15.9)	41.5 ± 3.5 (34–49)	121 ± 37 (30–205)	342 ± 54 (200–475)
26–40 weeks (49)*	13.7 ± 1.1 (11.8–17.3)	41.3 ± 3.4 (34–48)	120 ± 46 (45–280)	403 ± 58 (260–525)

* Number of subjects.

blood volume. This, however, is most unlikely, in view of the mass of published observations (at least among white pregnant women) to the contrary. A second and more likely possibility is that the increase in plasma volume as pregnancy progresses, is *quantitatively* paralleled by increases in total hemoglobin and total red cells. Whether this actually occurred must remain in doubt, since it was impracticable to carry out a series of determinations of blood volumes throughout pregnancy. Regarding this constancy of hemoglobin and hematocrit values, the following observations in the literature appear to be relevant to our subjects:

a. In the presence of a high iron intake, an otherwise inadequate diet does not prevent satisfactory hemoglobin production, even when chronic or acute hemorrhage occurs. Thus, in hypochromic anemia due to hookworm infestation, hemoglobin levels can be largely restored to normal limits by iron therapy alone, without either improvement in diet or removing the parasites from the intestinal tract (*22*, *23*). Furthermore, in poorly nourished Chinese blood donors, it has been found that hypochromic anemia can be prevented by iron therapy alone, without improving the diet consumed (*24*).

b. In non-anemic subjects with hemoglobin values within normal limits, a high iron intake can stimulate *increased* hemoglobin production. This elevation, not invariably observed, is usually transient (*19*, *21*, *22*, *24*).

c. In non-anemic pregnant women, it has been reported that a high intake can stimulate the production of hemoglobin and red cells, thus maintaining normal values throughout pregnancy (*25*, *26*).

Turning now to our Bantu subjects—their diet is inadequate (low in animal protein, fat, certain vitamins and mineral salts). But during pregnancy, and for years previously, their intake of iron has been very high. Their reserves of the element are thus probably unusually large. The presence of excessive reserves is verified frequently at *post mortem;* for example, in the Johannesburg area, Higginson, Gerritsen, and Walker (*13*) have noted that 50 per cent of adult females reveal abnormal deposition of iron in the tissues, often to a marked degree. The explanation of unchanged blood values in pregnancy would, therefore, seem to be the unusually high iron intake of our subjects.

Our finding of no significant change in serum iron value during pregnancy agrees with the observations of the American workers (*3*, *6*) and argues against the validity of the belief of Lundström (*5*) that a fall, *inter alia*, of serum iron in pregnancy, is "physiologic."

The rise we observed in total iron binding capacity is in harmony with other workers' findings (*2*, *4*, *5*). As far as we are aware, no explanation has been advanced to account for the phenomenon. There are two factors which *may be* of relevance. Firstly, iron is transported apparently exclusively by a β_1 globulin component of serum protein (*27*). Secondly, reduction in the albumin-globulin ratio reported to occur in pregnancy (*28*, *29*) suggests that the concentration of the particular iron binding globulin may be increased as gestation proceeds, although it forms only a small percentage of the total globulin fraction.

SUMMARY

The iron metabolism of the South African Bantu is marked by: (*a*) an habitually high iron intake; (*b*) occasionally elevated serum iron values; (*c*) extreme rarity of hypochromic anemia; and (*d*) abnormal deposition of iron in tissues of adult Bantu examined at necropsy.

Certain blood values have been determined on pregnant Bantu women, consuming a diet characterized by its very high iron content though otherwise inadequate. On comparing data in the first and second halves of the pregnancy period, it has been found that there is no significant change in values for hemoglobin, hematocrit, or serum iron. The constancy of these values would seem to be due to the unusually high iron intake of these people.

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BONE MARROW PRESSURE IN LEUKEMIC AND NON-LEUKEMIC PATIENTS

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It has been a frequent observation that many patients with leukemia and multiple myeloma bleed freely through the marrow needle at the time of bone marrow aspiration although hemorrhagic tendencies may not be present clinically. In addition, throbbing bone pain and bone tenderness are not infrequently observed in these patients (1). Such clinical observations suggest that some degree of pressure elevation may be present in the marrow cavity in these diseases. It was believed of interest to determine the pressure relationships within the human marrow cavity under a variety of physiologic stimuli.

MATERIALS AND METHODS

The marrow pressures of 14 patients with neoplastic diseases having essentially normal marrows, as determined by microscopic examination, and of 10 patients with leukemia and one patient with multiple myeloma were determined by means of recording strain gauges attached to clinical bone marrow needles. The patients were studied under resting but not basal conditions, without previous sedation. The pressure in both the sternal and iliac crest marrows was measured in most patients, either simultaneously or serially. The sternal site was at the level of the second interspace, and the iliac crest at a site two centimeters posterior to the anterior superior iliac spine. The usual precautions of sterility and local procaine anesthesia of skin and periosteum were employed.

A bonded SR4 wire resistance strain gauge and recording apparatus developed for clinical pressure measurements by Bierman and Jenkins (2) were employed for pressure determinations. The gauge was connected directly to the bone marrow needle after the needle and gauge chamber had been filled with heparin-saline solution. The saline within the manometer was equalized with the ambient pressure, after which the chamber was sealed. The output of the strain gauge was connected to a strain indicator and photographically recorded with a multiple channel recording oscilloscope.

The Turkel-Bethell bone marrow needle employed in these studies had a 14-gauge bore and was equipped with a trephine stylette which made it useful for removing bony spicules which frequently entered the needle during its implantation. After implantation, the needle was filled with heparin-saline solution, care being exercised to exclude air bubbles. It was found necessary to premedicate the patients with 50 to 100 mg. of heparin intravenously shortly before the studies to prevent clotting within the needle in the marrow. Smaller amounts of heparin were used in patients with leukemia, or completely omitted where manifest bleeding tendencies existed.

A modified strain gauge strapped over the lower chest cage was utilized as a pneumotachometer, and a single lead EKG tracing was made in each patient for pulse determinations. The marow pressure, respiratory rate and amplitude, and EKG record were simultaneously recorded photographically.

The marrow pressures were studied with the patients at rest in supine position, during exaggerated respiration, coughing, Valsalva and Mueller maneuvers, changes of body position, and following the injection of epinephrine. The Valsalva studies were roughly standardized by having the patients blow against a mercury manometer at 40 mm. Hg pressure.

Bone marrow for cytological examination was aspirated from each site studied at the conclusion of the pressure studies. An estimate of cellularity of the smears was attempted in each patient and recorded as normal, decreased, or increased, although it was recognized that this type of estimation may be markedly in error. The time required for the usual study averaged 30 to 45 minutes. Aside from the usual initial discomfort resulting from anesthetization of the skin and periosteum, and implantation of the marrow needle, no pain or discomfort was experienced by the patients during the pressure determinations.

RESULTS

The resting marrow pressures for all patients, with age, sex, diagnosis, hemoglobin level, and blood pressure, are shown in Table I. The patients can be divided upon the basis of the presence or absence of marrow disease. The marrows of the non-leukemic and lymphoma patients did not contain metastatic carcinoma or lymphomatous involvement, as ascertained by microscopic examination, and were of normal cellularity in all patients, except in J. K. where decreased cellularity was found. Most of the leukemic patients had increased cellularity of the marrow (Table I).

A. Non-leukemic marrows having no demonstrable tumor cells

The pressures in the marrows of patients without marrow diseases, including the lymphoma group, were found to be uniformly low. In the sternum the pressures were approximately atmospheric or slightly negative ranging from 2/0 to 17/15 mm. Hg. The sternal pressures frequently were observed to vary with the depth of respirations, while no effect of respirations was observed in the more distal iliac crest site. Figure 1 demonstrates a typical pressure recording made on the sternal and iliac crest marrow in a patient with Xanthomatosis, without cytological or peripheral hematologic evidence of marrow dysfunction.

The typical effect of the Valsalva maneuver is shown in Figure 1. In this patient both marrow sites demonstrated a prompt pressure rise when the patient expired against a mercury column of 40 mm. Hg. The usual pressure increase ranged from 10 to 60 mm. Hg. The Mueller maneuver, in most patients, resulted in a decided negative sternal pressure, varying from − 10 to − 30 mm. Hg and occurring concomitantly with the greater

TABLE I

Patient	Age	Sex	Diagnosis	Hemoglobin	Relative marrow cellularity*	Brachial artery cuff pressure	Sternal marrow pressure	Iliac marrow pressure
				Gm. %			*mm. Hg*	*mm. Hg*
O. H.	47	M	Carcinoma of lung	9.0	N	120/80	4/2	—
J. M.	51	M	Carcinoma of lung	10.7	N	105/70	−2/−3	11/10
M. M.	49	M	Epithelioma of lip	14.4	N	170/110	0/−1 to −5/−6	—
O. U.	40	M	Xanthomatosis	9.9	N	115/75	−1/−3	2/1
H. K.	44	M	Carcinoma of pancreas	11.2	N	110/80	—	10/5
G. R.	59	M	Melanoma	12.9	N	150/80	—	2/0
A. L.	47	M	Melanoma	8.9	N	147/85	—	5/2
A. B.	38	M	Melanoma	9.6	N	140/88	—	15/10
J. K.	63	M	Melanoma	14.7	D	120/80	—	17/15
I. R.	23	M	Lymphosarcoma	14.0	N	120/75	−2/−3	7/4
J. H.	68	M	Lymphosarcoma	13.7	N	125/80	0/−1 to −5/−6	8/5

* N: Normal cellularity.
I: Increased cellularity.
D: Decreased cellularity.

TABLE I—*Continued*

Patient	Age	Sex	Diagnosis	Hemoglobin	Relative marrow cellularity*	Brachial artery cuff pressure	Sternal marrow pressure	Iliac marrow pressure
				Gm. %			*mm. Hg*	*mm. Hg*
J. Z.	55	M	Lymphosarcoma	8.2	N	115/75	—	8/6
R. M.	23	M	Hodgkin's disease	7.9	N	120/70	—	4/0
R. W.	32	M	Acute lymphocytic leukemia	6.5	I	130/65	29/26	—
I. D.	41	M	Acute lymphocytic leukemia	9.0	I	145/90	21/0 to 10/−5	65/37 to 52/30
H. B.	24	F	Acute myeloblastic leukemia	10.6	I	110/65	—	63/45
R. R.	17	M	Acute myeloblastic leukemia	8.2	I	118/80	60/40 to 50/36	57/30 to 45/30
D. V.	16	M	Lymphatic leukemia	9.0	I	118/80	51/43	22/19
C. M.	73	M	Lymphatic leukemia	11.7	I	145/65	6/4 to 2/0	6/4
H. B.	68	F	Myelocytic leukemia	10.4	N	115/70	16/10 to 22/14	13/10
W. F.	67	M	Myelocytic leukemia	12.6	I	160/85	8/6	21/10
H. T.	54	M	Monocytic leukemia	10.7	N	120/80	—	15/6
R. J.	25	M	Monocytic leukemia	9.5	I	102/50	8/4	—
G. C.	57	F	Multiple myeloma	10.5	I	123/80	—	22/14

negative intrathoracic pressure produced by this maneuver. The Mueller effect was not reflected on the iliac crest pressure levels. Coughing produced decided alterations in the resting pressure. During the short inspiratory phase of the cough, a brief decrease in pressure was followed by a large brief pressure increase during the expiratory phase. The cough effect was most pronounced in the sternal site, but was frequently noted to a lesser degree in the iliac crest.

The typical effects on marrow pressure of altering body position are graphically shown in Figures 2 and 3. Readjustments in mean pressures and pulse pressure resulted from having the patient change from a recumbent position to a sitting position and again to recumbent.

Figure 3 was recorded while the subject was changing to the recumbent position from an upright position. A pronounced negative pressure resulted near the end of this act. The pressure

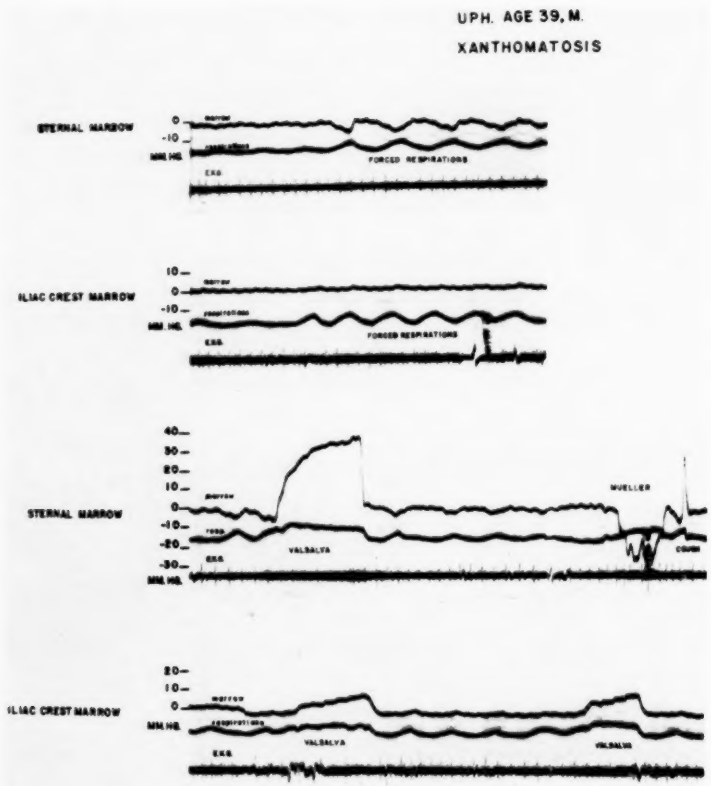

FIG. 1. RESTING PRESSURES IN MARROW OF STERNUM AND ILIAC CREST IN A PATIENT WITHOUT HEMATOLOGIC EVIDENCE OF MARROW DISEASE

Shown are the alterations in marrow pressure produced by forced respirations, Valsalva, and Mueller maneuvers.

then rose to a level 20 mm. Hg below that observed in the erect position. A progressive increase in iliac crest pressure was found to occur as the patient changed from a supine to sitting to standing and squatting positions.

The intravenous injection of 0.05 mg. epinephrine resulted in a decrease of mean pressure and diminution of pulse pressure. The sternal pressures became completely negative and showed the superimposed respiratory cycle. The iliac marrow also showed similar decreases in both mean and pulse pressures, which in no instance became negative. This effect of epinephrine was most pronounced in the leukemic subjects and is discussed below. The relative magnitudes of iliac marrow pressures and femoral artery pressure are shown in Table I in six patients.

Pulse wave analysis in the non-leukemic marrows revealed simple pulsations, except in the sternal sites where, in many patients, irregularities suggestive of venous pulsations were frequently superimposed upon the pulse waves (Figure 4, upper and middle tracings).

B. Leukemic bone marrow

The findings in this group of patients with leukemia revealed increased pressures in both marrow sites. The greatest mean pressures and pulse pressures were found in patients with the acute leukemias. Lesser pressure elevations were found in patients with other varieties of leukemia. The responses in leukemic marrow pressure to the Valsalva and Mueller maneuvers, cough, change of

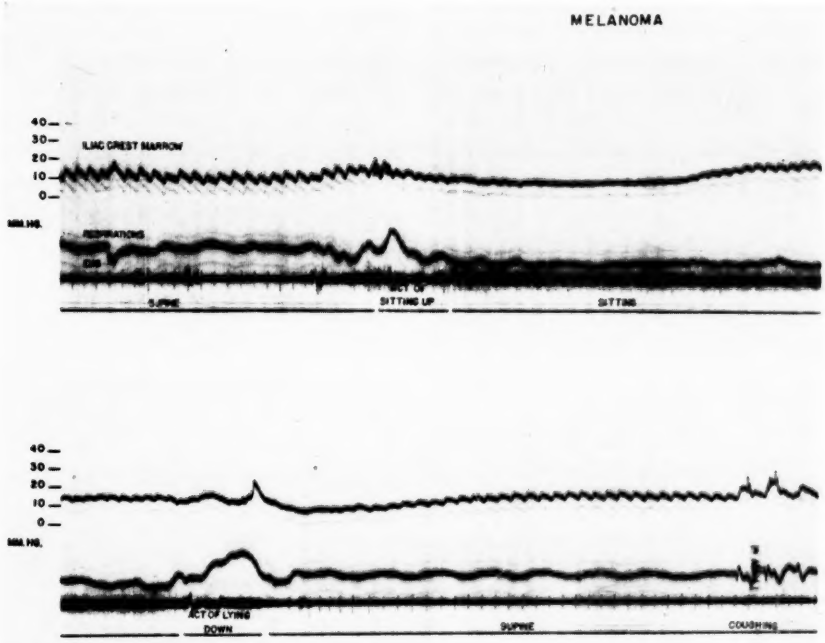

FIG. 2. ALTERATIONS IN ILIAC MARROW PRESSURE IN PATIENT A. B. CONTINUOUSLY RECORDED DURING CHANGE OF BODY POSITION

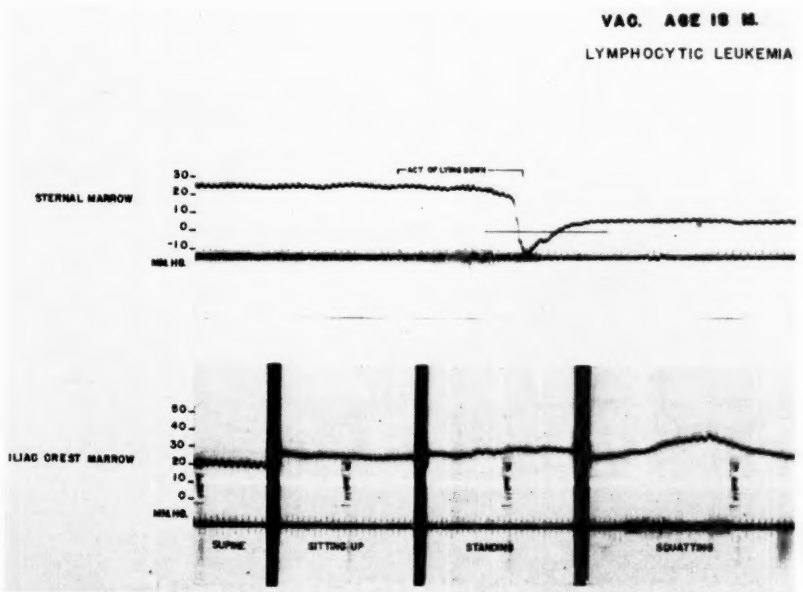

FIG. 3. ALTERATIONS IN MARROW PRESSURE PRODUCED BY BODY POSITION IN A PATIENT WITH LEUKEMIA

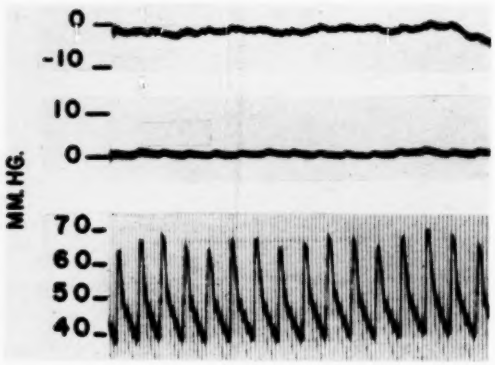

FIG. 4

position, and to epinephrine were similar in direction to those obtained in non-leukemic marrows, the difference being an exaggerated, more spectacular, response (Figure 5).

Analysis of the pulse waves in the leukemic group revealed dicrotic notching in those patients with the highest mean and pulse pressures. Figure 4 (lower tracing) shows the pulse pressure waves in patient I.D. with acute monoblastic leukemia.

There was no apparent correlation between the mean pressures and pulse pressures in the marrow and the degree of anemia within the range of hemoglobin levels in these patients (Table I).

DISCUSSION

Larsen (3) found the average pressure in the long bones of dogs to be 30 to 40 mm. Hg, and found that intravenously administered epinephrine, pitressin, and histamine resulted in a reduction of the total marrow pressure, while the administration of ephedrine resulted in a rise in marrow pressure. Poa (4) demonstrated the vasoconstricting effect on the marrow of epinephrine and sympathetic nerve stimulation in the long bone of the dog. Rasgon, Vater, and Marbarger (5), employing the strain gauge on long bones in dogs, con-

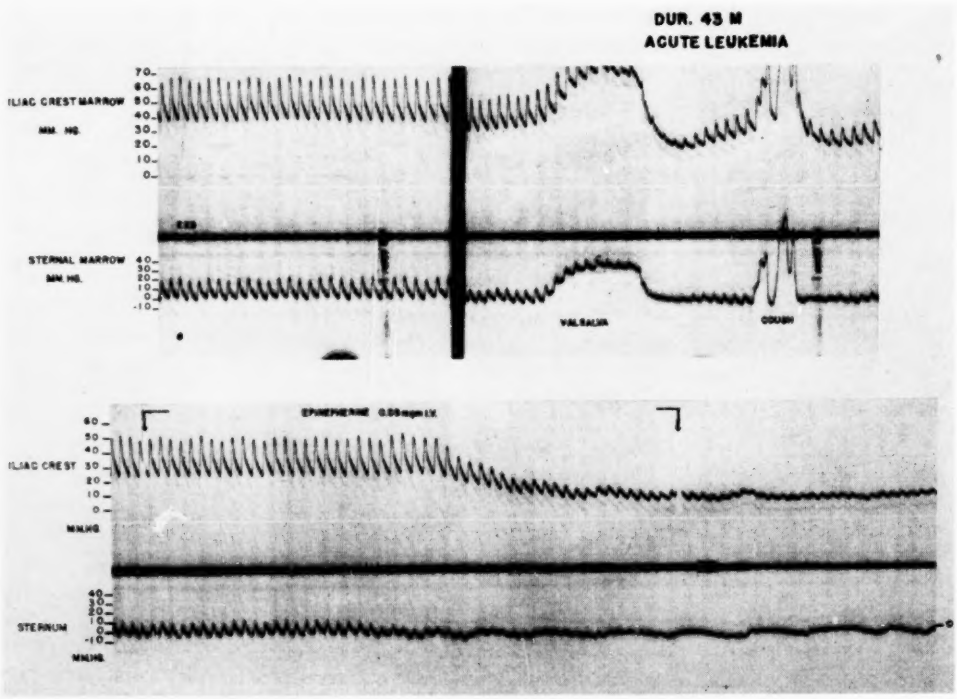

FIG. 5. UPPER FIGURE: TYPICAL PRESSURE RECORDING FROM A PATIENT WITH ACUTE MONOBLASTIC LEUKEMIA LOWER FIGURE: DEPICTING THE EFFECT OF 0.05 MG. INTRAVENOUSLY ADMINISTERED EPINEPHRINE ON ILIAC CREST AND STERNAL MARROW PULSE PRESSURES

cluded that the marrow behaves as a semi-closed cavity and that changes in the intramedullary pressure are dependent upon the volume of blood within the marrow cavity. When the venous return is obstructed, the mean pressure of the marrow increases and the pulse pressure decreases, whereas compression of the afferent arterial blood supply, mechanically or by epinephrine vasoconstriction, decreases both the mean and pulse pressures. Kalser and his co-workers (6) demonstrated a direct correlation between altitude and a fall in marrow pressure in dogs, and experimentally confirmed that the marrow cavity acts as a semi-closed cavity.

Data obtained in the present study support the interpretation of Rasgon and Kalser that the marrow cavity physiologically resembles a semi-closed cavity. The result of the Valsalva maneuver is a rise in the systemic venous pressure, thus reducing the venous outflow from the marrow cavity and producing an increase of mean marrow pressure and decreased pulse pressure. Epinephrine decreases the afferent blood flow to the marrow by constricting the nutrient arterioles, and produces a decrease in total pulse pressure in the bone marrow. The human marrow pressures were generally much lower than those found in dogs, possibly due to the effects of ether or barbital anesthesia in the dogs.

This study of respiratory alterations and of body position demonstrates the lability of the human bone marrow pressures as a result of such changes. Sternal pressures were found to be near or below atmospheric pressures in recumbent non-leukemic patients, and to vary with the amplitude of respirations. The more distal iliac sites did not respond to the respiratory effects of forced breathing, but required the more strenous effects of the Valsalva effort or coughing. The higher pressures obtained in the iliac crest are presumably a result of the erect position of man. In the patients, the mean iliac crest pressure rose with the degree of erectness, while the pressures were more variable in the sternum, and in all cases the increases in pressures were much less than in the ilium. The effects of body position and of respiratory alterations on marrow pressures indicate that under conditions of normal activity the intra-medullary pressure varies considerably and is passively affected by changes in the venous pressure resulting from these activities.

The patients with leukemia and multiple myeloma differed from non-leukemic patients in having elevated mean pressures and increased pulse pressures in the marrow cavities. In the patients with acute leukemia where the highest pressures were found, dicrotic notches were present in the pulse waves suggesting a lowered peripheral resistance in the marrow circulation in these patients. The degree of anemia present in these patients could not be correlated with the mean pressures or the pulse pressures in the marrow. These pressure data confirm the increased vascularity in the marrow in some forms of leukemia as has been previously demonstrated by the clearance of I^{131} from the marrow (7).

In many patients with multiple myeloma and metastatic bone lesions, distinct bone pain is associated with sudden straining or coughing. It is possible that the sudden elevation of intramedullary pressure shown to result from such effort results in distortion of the arteries and arterioles bearing sensory nerves and thus produce pain. It is also conceivable that long-continued exaggerated intramedullary arterial pulsations, in a manner analogous to the arterial notching of ribs in coarctation of the aorta, may contribute to erosion of the trabeculae frequently observed in leukemic bone marrow.

Practically no information is available regarding the hematopoietic effects of such alterations in marrow pressures as is seen in physiological and pathologic states. Drinker, Drinker, and Lund (8) were unable to dislodge cells from the marrow of dogs by continuous perfusion. Foa (4) however demonstrated that 15 minutes following the vasoconstrictive effect of epinephrine, an increase occurred in the total number and the percentage of immature cells in the venous blood draining the marrow of dogs. Comparable studies in man are lacking, but the factor of abrupt alterations in marrow pressure resulting from normal physiologic activity merits further consideration as a mechanism of blood cell delivery from the marrow in addition to such postulated factors as growth pressure and level of cell maturity (9, 10). The concept that the marrow tissues are sheltered from external forces in a bony cage where constant en-

vironmental conditions prevail is not supported by the findings in the present investigation.

SUMMARY

The sternal and iliac crest bone marrow pressures of patients having neoplastic diseases and patients with leukemia were determined by means of recording strain gauges. The responses in marrow pressure were determined under a variety of physiologic stimuli. The human marrow circulation was found to be readily influenced by normal physiological variations of respiration and body position.

The alterations produced in marrow pressures in leukemic and non-leukemic patients were similar. However, markedly elevated pressures were found in leukemic patients. The significance of the findings was discussed in terms of normal and leukemic bone marrow physiology.

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FREE BLOOD CORTICOIDS IN THE NEWBORN INFANT[1]

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Infants in the first week of life respond to ACTH administration with a smaller fall in circulating eosinophils and a smaller increase in urinary corticosteroids than these same or other infants do when they are older (1, 2). Since these findings seem to validate the suggestions that certain infants suffer from transient adrenal failure and that others are unable to withstand the stress of surgery in the newborn period because of a lack of adrenal cortical hormones (3, 4), the logical inference is that these infants should be treated with adrenal cortical hormones. The administration of these hormones to infants is dangerous. In this period particularly, in addition to all the potential dangers they possess in older children and adults, they inhibit growth, increase susceptibility to infection, and are associated with a high mortality (5, 6).

It is important, therefore, that two questions be answered. Do newborn infants have a period of transient adrenal hypofunction and if so, does this have any clinical significance? This study attempts to answer the first question.

Up to this time the evidence that the newborn infant has a period of depressed adrenal function for approximately a week has been indirect. Corticosteroid excretion is affected by urinary clearance rates and methods and rates of hormonal degradation, etc. Recently, Bergstrand, Hillström, and Jonsson have challenged the validity of the eosinophil measurements as evidence (7). The present study was undertaken to make direct measurements of adrenal corticoids in the serum of normal newborn infants.

MATERIALS AND METHODS

The method of Nelson and Samuels for measuring blood 17 hydroxycorticoids utilizing florisil as the chromatographic agent was used throughout (8)[3]. The only change in the method as published was the use of serum instead of plasma for most of the measurements in the present study. The serum or plasma of 41 children and adults considered normal for the purpose of this study was analyzed for control measurements. The ages of these subjects ranged from 3 weeks to 32 years. The group included 13 infants under one year of age. The adults were laboratory workers in apparent health. The children either were in the hospital for elective surgery, for the investigation of chronic neurologic disorders or behavior problems, or had recovered from minor respiratory infections. Fourteen specimens obtained from newborn infants from two days to five days of age were analyzed. The deliveries of these infants were normal, and the infants were considered healthy by the examining pediatricians. Since the method requires 10 ml. of serum or plasma, it was necessary to pool sera from two infants in eight instances. In addition, serum was obtained from each of six children with erythroblastosis fetalis before exchange transfusion was carried out. One specimen of 10 ml. of serum was obtained from a seven-day old normal newborn infant. Sera were also obtained from 23 cord bloods of normal children born of normal deliveries.

RESULTS

The mean level of circulating compound F-like substance in the blood of normal children and adults was 13 micrograms per 100 milliliters of serum. The standard error of the mean was ± 1.21. This agrees with the mean of 13 μg. per 100 ml. reported by Nelson for normal adults and by Kelley and Ely for normal children (9). There was no discernible difference in the various age groups within this overall group of 41 normal individuals, 3 weeks to 32 years of age. Figure 1 graphically shows the results found in the control subjects, the cord bloods and the normal newborn infants. The mean level in the 23 cord bloods was 21.4 micrograms per 100 milliliters of serum. If the two unusually high results of 100 and 57 micrograms per cent are excluded, the mean level is 16.0. In any event, these results are not significantly different from those found in the normal

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[3] Standard compound F was kindly supplied by Dr. W. J. Haines, Upjohn Company.

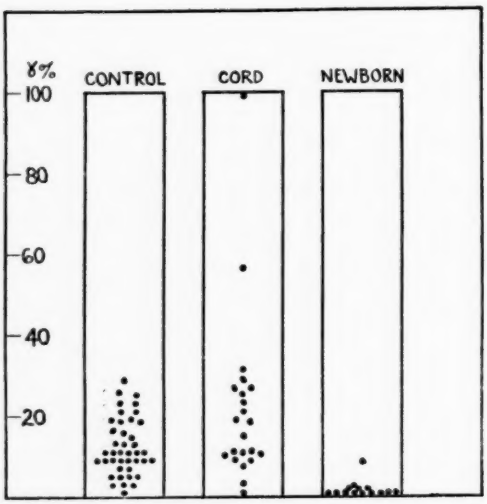

FIG. 1. FREE CORTICOIDS IN SERA OF 41 NORMAL CHILDREN AND ADULTS, THREE WEEKS TO 32 YEARS OF AGE: 23 CORD BLOOD OBTAINED FROM NORMAL INFANTS BORN OF NORMAL DELIVERIES: 14 NORMAL NEWBORN INFANTS TWO TO FIVE DAYS OF AGE

control subjects. The mean level found in the 14 normal infants was 0.9 with a standard error of the mean of ± 0.6. As may be seen by inspection there is only one level above 2 micrograms per cent. Actually any level below 2 micrograms per cent probably should be considered a zero measurement (10). The one specimen obtained from a one week old child contained 15 micrograms per 100 milliliters of serum. The results obtained from six patients with erythroblastosis were as follows: 16, 12, 2.5, 1, 0, 0 micrograms per 100 milliliters. The age and weight of these patients are shown in Table I. Acid hydrolysis of the sera from four normal newborn infants showed no significant increase in the amount of compound F-like substance measurable.

TABLE I

Age, sex, birth weight, and level of free serum compound F of six children with erythroblastosis fetalis before exchange transfusions

Pt.	Sex	Age	Birth wt.	"F"
		Hrs.	*Kg.*	*γ%*
TH	M	12	2.80	12.0
JA	F	30	2.95	15.9
JO	M	4	2.95	0
HA	M	23	2.60	1.0
WI	M	8	3.15	0
BA	F	5	2.60	2.5

DISCUSSION

The uniform finding of absent or very small amounts of compound F-like substance in the serum of normal newborn children bears out previous indirect evidence of a period of lowered adrenal function in the newborn period. The normal levels in the cord blood samples probably represent maternal hormone. The more variable results obtained from the sera of children with erythroblastosis were to be expected and presumably represent different rates of degradation or excretion of the maternal hormone. It was surprising to the authors that so many low levels were obtained so soon. The obvious difficulties in obtaining specimens have made it impossible to determine the time at which the average newborn infant first has a normal circulating level of free 17 hydroxycorticoids but from previous indirect evidence it might be assumed that this would happen sometime in the second week of life.

Jailer has shown that the newborn rat pituitary produces very little ACTH (11). ACTH increased in the newborn rat pituitary with the passage of time and simultaneously with this the adrenal becomes more responsive.

The excretion of 17-ketosteroids in the urine of the normal newborn infant is high in the first day or two and then gradually drops to the low levels of later infancy (12). Gardner has shown similar changes in blood ketosteroids (13).

From the work of Gardner, the present work and the urinary excretion studies it is apparent that maternal steroids are passed to the fetus and that these rapidly disappear in the neonatal period. This fact coupled with the logical inferences from Jailer's work would seem to strengthen the original hypothesis advanced by one of the authors in 1950 that the period of lowered adrenal responsiveness in the newborn is due to suppression of pituitary production of ACTH by the excess maternal corticoids passed transplacentally (1).

It must be emphasized, however, that it has not been demonstrated, as yet, that this period of relative adrenal hypofunction does the child any harm. The hypofunction is relative and the adrenal can respond to sufficient stimulus (2, 3).

SUMMARY

Sera have been analyzed for free compound F-like material from normal infants and adults, from cord bloods from normal children born of normal deliveries, from 14 normal newborn infants and from five infants in the first day of life with erythroblastosis. It has been shown that the normal newborn infant from two to five days of age has a significantly lower level of circulating free compound F-like substance than have normals from the age of three weeks to 32 years. Indeed most of the newborn infants have no measurable levels. Cord bloods have amounts of free compound F-like substances that approximate the amount circulating in normal adults. The levels obtained from children with erythroblastosis in the first day of life vary greatly and are intermediate between the values found in cord bloods and in normal newborns of two to five days of age.

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THE TRANSPORT OF RIBOFLAVIN BY HUMAN PLACENTA[1]

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The human placenta has been shown to have the ability to transfer thiamin (1), ascorbic acid (2–4), fructose (5), organic and inorganic phosphate (6), and, early in pregnancy, glucose (6) from maternal to fetal blood. No data were available concerning the concentration of riboflavin in normal maternal and fetal bloods. Although some analyses had been performed of the riboflavin content of tissues from fetal and stillborn infants (cf. Smith [7]), the riboflavin content of the placenta was unknown. In this paper are presented the results of riboflavin analyses of ten normal placentas and of maternal and cord blood obtained during ten normal deliveries. These analyses suggest that an active metabolic process is involved in the transfer of riboflavin from maternal to fetal blood by the placenta.

METHODS

Samples of maternal blood were collected by venepuncture during full term delivery and samples of cord blood were obtained immediately after the expulsion of the placenta. The blood samples were permitted to clot normally and then centrifuged to obtain the serum. The serums were analyzed by the method of Burch, Bessey, and Lowry (8), in which separate estimations of free riboflavin (FR), riboflavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD) are made. The serums were diluted ten-fold with 5 per cent trichloroacetic acid for analysis. The placentas from these deliveries were also analyzed for free riboflavin, riboflavin mononucleotide and flavin adenine dinucleotide by the method of Bessey, Lowry, and Love (9). One Gm. of placenta was minced in a Waring blendor with 50 ml. of redistilled water and then diluted with an equal volume of 20 per cent trichloroacetic acid.

The determinations of riboflavin were made using a Farrand photofluorometer[2]; fluorescein standards of 2.5 and 10 millimicrograms per ml. were used. The analyses were carried out in the dark at 5° C. and were made in duplicate or triplicate.

RESULTS

The results of the analyses of serum are given in Table I. Fetal (cord) blood at term has a significantly higher concentration of total riboflavin than maternal blood. This is due to the fact that fetal blood has about four times as much free riboflavin as maternal blood yet only half as much flavin adenine dinucleotide. The amounts of riboflavin mononucleotide (FMN) in both maternal and fetal bloods are very small. The analyses of maternal blood are comparable to those found by Burch, Bessey, and Lowry (8) for normal human blood.

Analyses of placenta showed that it had an average of 214 micrograms total riboflavin per 100 Gm. (wet weight) tissue. Of this 205 micrograms were FAD and only 9 were FMN plus free riboflavin. This is comparable to the amounts found in rat brain and skeletal muscle by Bessey, Lowry, and Love (9). These investigators found much higher concentrations of riboflavin in rat liver, kidney and heart, but in all tissues, 80 per cent or more of the riboflavin was present as FAD.

The results of the analyses of serum suggested that neither free riboflavin nor FAD can diffuse readily across the placenta and that the placenta maintains the higher concentration of riboflavin in fetal blood by splitting FAD to free riboflavin. To determine whether the placenta had enzymes for splitting FAD, minced placenta was incubated in a modified Ringer's solution (90 mM per L. Na^+, 50 mM per L. K^+, 10 mM per L. Mg^{++}, and 40 mM per L. phosphate), to which was added enough FAD so that the initial FAD concentration was 300 micrograms per 100 Gm. placenta. One Gm. of placenta was minced and incubated at 37° C. for one hour with shaking in 100 ml. of solution contained in 250 ml. Erlenmeyer flasks.

The results of analyses for FAD, FMN, and free riboflavin before and after incubation are given in Table II. It is clear that the placenta has a mechanism, presumably enzymatic, for

[1] This investigation has been aided by a grant from the Charles A. King and Marjorie King Fund and by an institutional grant from the American Cancer Society.

[2] We are greatly indebted to Dr. Clement A. Smith for his kindness in permitting us to use his Farrand photofluorometer.

TABLE I

Riboflavin concentrations in maternal and fetal serum expressed in micrograms per 100 ml. of serum

Sample	Free riboflavin		Flavin adenine dinucleotide		Riboflavin mononucleotide		Total riboflavin	
	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal
1	1.36	3.17	3.00	2.20	0	0.1	4.10	5.40
2	0.68	2.25	2.31	1.74	0.3	0.25	3.29	4.25
3	0.26	2.19	2.70	0.85	0.04	0.6	3.00	3.65
4	0.67	2.49	3.18	1.99	0	0	3.87	4.47
5	0.25	2.24	2.08	0.83	0.04	0.5	2.37	3.53
6	0.64	2.77	3.33	0.95	0	0.45	3.80	4.15
7	0.18	1.76	1.86	1.09	0.31	0.17	2.35	3.02
8	0.56	1.46	2.25	1.11	0	0.26	2.75	2.83
9	0.50	1.42	1.70	0.96	0.09	0.17	2.29	2.55
10	0.35	1.63	2.34	1.27	0.16	0.28	2.85	3.18
Mean	0.55	2.14	2.48	1.29	0.09	0.28	3.07	3.70
Standard error	±0.12	±0.18*	±0.18	±0.16*	±0.10	+0.15	±0.20	±0.29†

* p 0.01 and † p 0.05 that the fetal-maternal differences are not significant, calculated by paired t test.

splitting FAD to FMN and FR and that the amount of FMN and FR formed accounts for the amount of FAD that disappeared.

DISCUSSION

These experiments show that there is significantly more free riboflavin and significantly less FAD in fetal blood than in maternal blood. The total amount of riboflavin (FAD + FMN + FR) is greater in fetal blood than in maternal blood.

These analyses also show that placenta contains large amounts of riboflavin and that, as in other tissues, most of this riboflavin is present as FAD. Minced placenta incubated *in vitro* will split FAD to FMN and FR.

These data suggest that the placenta transfers riboflavin from maternal to fetal blood by taking in riboflavin from the maternal blood as FAD, splitting the FAD and secreting the free riboflavin into the fetal blood. An alternative explanation, that fetal tissues can synthesize the isoalloxazine ring and thus increase the concentration of free riboflavin in fetal blood, seems unlikely. Presumably, fetal tissues need FAD, not free riboflavin, and synthesize their own FAD from the free riboflavin taken in from the fetal blood. In the course of these experiments one sample of 20-week fetal liver became available. It was found to contain 670 micrograms of total riboflavin per 100 Gm. wet tissue, almost all of which was FAD. This finding is in accordance with the hypothesis that fetal tissues synthesize their own FAD from riboflavin of the blood.

TABLE II

*The conversion of flavin adenine dinucleotide to flavin mononucleotide and free riboflavin by minced human placenta**

	Initial	Final	Change
Total riboflavin	362	383	+ 21 ± 7
Flavin adenine dinucleotide	292	169	−123 ± 13
Free riboflavin	8	56	+ 48 ± 3
Flavin mononucleotide	62	161	+ 99 ± 14

* Values are the averages of three experiments, expressed as micrograms per 100 Gm. (wet weight) placenta, ± the standard error of the mean change.

SUMMARY

1. Photofluorometric analyses of maternal and fetal serum demonstrated that the concentration of free riboflavin is about four times as great in fetal blood as in maternal blood; in contrast, the concentration of flavin adenine dinucleotide is about twice as great in maternal blood as in fetal blood. The total concentration of all forms of riboflavin, free, mononucleotide and dinucleotide, is greater in fetal than in maternal blood.

2. Minced placenta incubated *in vitro* will split FAD to FMN and free riboflavin.

3. The data suggest that the placenta transfers riboflavin from maternal to fetal blood by taking in FAD from the maternal blood, splitting this to free riboflavin, and secreting the free riboflavin into fetal blood.

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THE RESPIRATORY DEAD SPACE MEASURED BY SINGLE BREATH ANALYSIS OF OXYGEN, CARBON DIOXIDE, NITROGEN OR HELIUM[1]

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Fowler (1) estimated the respiratory dead space in man by recording simultaneously and continuously the nitrogen concentration and the volume flow at the mouth during a single expiration, following a breath of oxygen. Such an expiration may be divided into three phases. In the first part of expiration there is virtually no nitrogen. This is followed by an S-shaped rise in nitrogen concentration as alveolar gas begins to appear. Finally, when the dead space is washed out, the third phase, the 'alveolar plateau' is recorded. By substituting for the S-shaped curve a vertical line which represents a theoretical square front between dead space air and alveolar air, and computing the expired volume up to this front, Fowler obtained an estimate of the dead space for nitrogen, defined as "the volume of the conducting airway down to the location at which a large change in gas composition occurs."

The respiratory dead space for oxygen and carbon dioxide is of interest for several reasons. Firstly, the dead space presumably includes upper airways, bronchi and bronchioles. It is possible that there may be an appreciable transfer of oxygen and carbon dioxide between gas and blood in the smaller bronchioles, though not for the relatively insoluble gas nitrogen; in this case, these fine tubes would no longer be "dead space" for oxygen and carbon dioxide, so that the dead space volume for these gases would be smaller than for nitrogen. DuBois, Fowler, Soffer, and Fenn (2) measured the respiratory dead space for carbon dioxide but did not make comparisons of it with that for other gases in their subjects. Pappenheimer, Fishman, and Borrero (3), using an entirely different approach, recently showed that the dead space for carbon dioxide was the same as that for oxygen. They also made no comparisons with other gases.

Secondly, Krogh and Lindhard (4) and Douglas and Haldane (5), making measurements upon "alveolar" carbon dioxide, obtained vastly different results for the respiratory dead space. Haldane believed that a representative alveolar sample could only be obtained at the end of a forced expiration, and that samples collected earlier in expiration were contaminated by dead space gas. Krogh and Lindhard showed that in the later stages of a single expiration, the concentration of carbon dioxide, when plotted against time, rose to a plateau. They believed that an expired volume of about three times the dead space was sufficient to wash out the dead space completely and that subsequent changes in concentration were due to variations in the composition of alveolar gas itself. Fowler (6) confirmed the approximate straight line characteristic of the 'alveolar plateau' when he analyzed nitrogen continuously in the expired air during a single breath. He showed that once the nitrogen concentration curve reached the alveolar plateau, the changes in concentration occurring thereafter, if any, were due to uneven ventilation and not to further mixing of alveolar gas with dead space gas. The availability of a mass spectrometer for rapid gas analysis enabled us to make measurements of the alveolar plateau and of the dead space for oxygen, carbon dioxide, nitrogen and helium. With the possible exception of the helium dead space in some subjects, we have been able to demonstrate not only that the various dead spaces are the same, but also that the same volume of expired gas is required to completely wash out the dead space for oxygen, carbon dioxide or nitrogen.

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[2] Medical Research Fellow of the National Tuberculosis Association.

METHODS

A. Instruments. To study the changing concentration of various gases present in a breath during expiration, we used a mass spectrometer [3] which continually samples any stream of gases that is led past it, by drawing 8 ml. of gas per minute through a metal tube of 0.006 inch bore. The output was led through a DC amplifier to a magnetic pen writer. There was a latency interval of 0.06 second due primarily to the time needed for the gas to traverse the sampling tube and enter the analyzer. Another 0.06 second was then required for 90 per cent response, resulting from the delay in the electrometer circuit and washout of the various chambers of the instrument. The analytic error of the system was less than 1 per cent of full scale deflection on the record. The output was linear, and concentrations of less than 0.05 per cent oxygen, 0.04 per cent carbon dioxide, 0.05 per cent nitrogen and 0.25 per cent helium could be detected. The resolving power and other characteristics of the instrument made the output independent of other gases present in the sample, within the limits required by these experiments.

In a number of experiments, we used a Lilly nitrogen meter as well as the mass spectrometer, to obtain simultaneous and continuous records of expired carbon dioxide, or oxygen, with nitrogen. The accuracy of this meter was ± 2 per cent of full scale deflection. It had a latency interval of 0.06 second, required another 0.03 second for 90 per cent response and sampled at the rate of 12 ml. per minute.

It was necessary to make accurate measurements of volume flow for only approximately 400 ml. of expired gas. Therefore we used a 1 liter spirometer with a 70 Gm. bell. To the shaft of the pulley was attached a 10,000 ohm wire wound low torque potentiometer [4] with a 1½ volt battery across it. The contact voltage was led through a DC amplifier to a magnetic pen writer. The pen deflection was a linear function of spirometer bell displacement, and gave a continuous record of the first 400 ml. of expired gas. The system was accurate within 1 per cent of full scale at flow rates up to at least 115 liters per minute. The 90 per cent response time was less than 0.02 second.

Initially, we used a Lilly flow meter to estimate expired volume. However, the integrations were laborious, and in addition, the Lilly flow meter was affected by condensation of water on the screen to such an extent that its sensitivity to flow was appreciably changed by the water vapor in one breath. Although this effect could have been corrected, it was simpler to use the spirometer which is not influenced by water vapor, and which yields a volume record that does not require further calculations.

Inspired volume was recorded on an ordinary 6 liter spirometer which was filled with air, 100 per cent oxygen, 80 per cent helium–20 per cent oxygen, 100 per cent nitrogen or 100 per cent nitrous oxide, depending on the gas we wished to study during expiration.

[3] Consolidated Engineering Corporation, Pasadena, California.

[4] Helipot Company, South Pasadena, California.

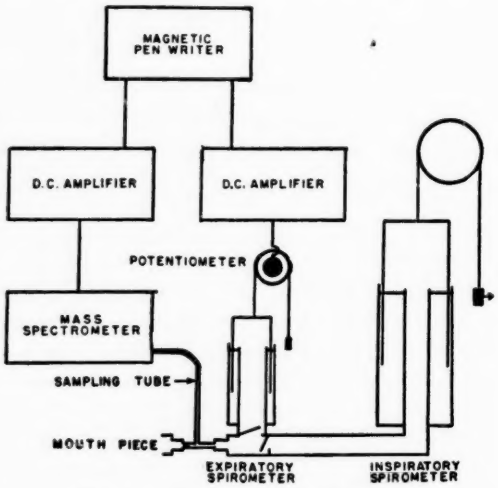

FIG. 1. ARRANGEMENT OF APPARATUS

The arrangement of apparatus is shown in Figure 1. The sampling tubes of the mass spectrometer and of the nitrogen meter were fitted in the mouth piece. The instrumental dead space, from the edge of the mouthpiece to the tip of the sampling tubes, was 9 ml. In order to obtain an average sample from the gas stream, we found it necessary from experiments on model systems, to constrict the lumen of the mouthpiece, stepwise, to about 6 mm. diameter at the site of connection with the sampling tubes. At the expiratory flow rates used, the pressure across this constriction was 0.5 cm. of H_2O or less. We routinely made a correction of 0.09 second for the lag of the mass spectrometer, regardless of the speed of concentration change, because we found from the same experiments on models that this single correction figure combined convenience and accuracy within the limits of error of the method.

B. Subjects. All were research workers, trained to

TABLE I

Physical characteristics of subjects

Subject	Age (*years*)	Height (*feet and inches*)	Weight (*lbs.*)
1	34	5–6	140
2	32	6–2	165
3	30	5–10	145
4	33	5–10	180
5	31	6–2	185
6	34	5–11	160
7	27	5–7	150
8	33	6–4	200
9	35	5–11	150
10	35	6–5	190

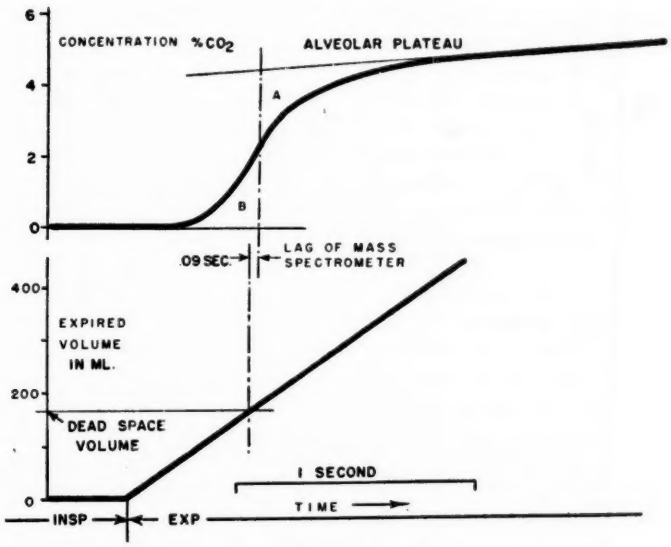

FIGURE 2

In this schematic representation, the upper part of the record is from the mass spectrometer, the lower part from the light-weight one liter spirometer with attached potentiometer. A straight line is drawn through the 'alveolar plateau.' A vertical line is then drawn so that area A equals area B. This shows the time at which the transition from dead space gas to alveolar gas would have occurred if the alveolar gas had come through the mouth as a square front. As we were interested in the first part of the expired breath only, the gain of the potentiometer recording system was turned up so high that after about 400 ml. were expired, the pen writer ran off scale.

control their breathing. Their physical measurements are given in Table I. Subject 1 was a woman, the others were men. Subject 1 had a therapeutic unilateral pneumothorax about eight years prior to these experiments. Subject 10 had a pleural effusion twelve years prior to these experiments. Otherwise, all subjects were in good health.

C. Procedures. The subject always sat in the same position and wore a noseclip. From his normal end-expiratory level, he took a breath of 300 to 900 ml. from the 6 liter spirometer. After holding his breath with closed glottis for periods ranging from 1 to 60 seconds, counted from beginning of inspiration to beginning of expiration, he expired evenly into the 1 liter spirometer. Breathholding times of 5 seconds or less were measured to the nearest 0.04 second on the mass spectrometer record. Longer breathholding times were measured with a stopwatch. A continuous record of gas concentration and of the first part of the volume flow was made during this expiration. Although only the first 400 ml. were graphically recorded, the subject expired more than 1 liter at a steady rate, the excess escaping around the spirometer bell.

RESULTS

Figure 2 shows schematically a typical record of expired gas concentration and volume. The theoretical boundary separating dead space and alveoli was found by Fowler's 'estimation' technique (1) which assumes that although the flow rate varies markedly during expiration it does not change greatly during the fraction of a second when the gas concentration is changing most rapidly. The inaccuracy introduced by this technique is less than the other errors of measurement. After correcting for time lag of the mass spectrometer, the amount of gas which has passed the sampling tip of the mass spectrometer up to the theoretical boundary is determined by dropping a perpendicular line to the volume record. From this value, 9 ml. is subtracted (dead space of mouthpiece) and the resulting figure, corrected to

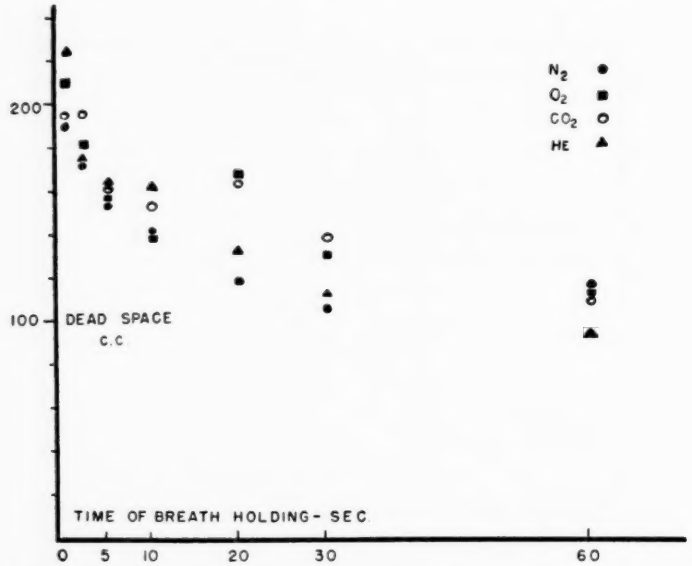

FIG. 3. DECREASE OF ONE SUBJECT'S DEAD SPACE VOLUME VERSUS TIME OF BREATHHOLDING FOR FOUR DIFFERENT INDICATOR GASES

All inspirations were 80 per cent helium–20 per cent oxygen.

BTPS, is the dead space volume for the indicator gas in question.

The data for Subject 3 are shown in Figure 3; the volume of the dead space is plotted against the time of breathholding, for four different indicator gases. Each point is the average of 2 to 10 observations. It is evident that the dead space for all four indicator gases decreases with increasing time of breathholding in the same manner. We do not feel that the unusually large dead space volumes for oxygen and carbon dioxide after 20 seconds of breathholding, seen only in this subject during this experiment, have any special significance. The standard error of any point on the graph was as high as ± 20 ml. With the exception noted above, this curve was typical for those subjects (2, 3, 4, 6, and 10) in whom similar observations were carried out. There was no significant difference in the rate of decrease of dead space volume with time of breathholding among the different indicator gases.

The scatter of the points seemed to be the same for the various times of breathholding. In an effort to reduce this scatter, we standardized the conditions of the experiment more rigidly, and made a large number of observations on Subjects 1, 5, 6, 9, and 10, at 2½ seconds of breathholding for all four indicator gases: oxygen, carbon dioxide, nitrogen, and helium. The inspired gas was 80 per cent helium–20 per cent oxygen throughout. The subjects grasped the mouthpiece, which consisted of a metal tube, with their lips only, breathing through clenched teeth. This detail was necessary because we observed that allowing the teeth to be separated could produce an increase in dead space of 50 to 100 ml. as shown by Lindhard (7). Table II shows that there were no consistent differences in volume of dead space in Subjects 6, 9,

TABLE II

*Dead space (ml.) of five subjects for four gases**

Subject	O_2	CO_2	N_2	He
1	161 ± 8	144 ± 4	134 ± 6	124 ± 5
5	149 ± 4	145 ± 4	141 ± 5	128 ± 3
6	169 ± 9	161 ± 6	152 ± 4	165 ± 3
9	165 ± 5	160 ± 5	155 ± 6	169 ± 3
10	195 ± 5	207 ± 7	198 ± 9	189 ± 5

* All breathholding times were about 2½ seconds. All inspirations were 80 per cent helium–20 per cent oxygen, and followed a period of breathing air. The figures are averages and standard errors of the mean of 3 to 20 observations, and are corrected to BTPS. Subjects had their teeth clenched.

and 10, for the four indicator gases used. The differences shown by Subjects 1 and 5 will be discussed below.

To determine the effect of breathing helium on the oxygen and carbon dioxide dead spaces, we compared in subjects 1, 4, 5, 6, 9, and 10 the dead space of these two gases following: (a) A breath of air; (b) a single breath of 80 per cent helium–20 per cent oxygen; and (c) a three minute washout of the alveoli with this same mixture. We found no consistent difference.

In the experiments described so far, the dead space for the four indicator gases had to be measured on different breaths since our mass spectrometer will record only the concentration of one gas at a time. However, if the nitrogen meter is used to analyze for nitrogen while the mass spectrometer analyzes for oxygen or carbon dioxide, it is possible to obtain a continuous record of the concentration of two gases during the same expiration. This method was used on Subjects 5, 6, 7, 8, and 10. The subject inspired one breath of either 100 per cent nitrous oxide or 100 per cent oxygen, held his breath for 2½ to 30 seconds, and expired through a mouthpiece fitted with sampling tubes leading to both the mass spectrometer and a nitrogen meter. One hundred per cent oxygen was inspired when nitrogen and carbon dioxide concentrations were to be measured during expiration, and 100 per cent nitrous oxide when nitrogen and oxygen were to be measured. Nitrous oxide was selected by elimination. Among the available gases, nitrogen and oxygen could not be used because the dilution by water vapor of a dry inspired gas in the dead space produced a stepwise decrease in the record of the expired gas concentration which confused interpretation, if this same gas was used as the indicator. Helium could not be used because it distorts the N_2 meter output.

To compensate for the differences in absolute value and slope of the respective alveolar plateaus, the expired gas concentrations at each point were recalculated as percentages of the extrapolated 'alveolar plateaus' at that point. As shown in Figure 4, the corrected nitrogen concentration from the nitrogen meter record, and the corrected oxygen or carbon dioxide concentration from the mass spectrometer record always described virtually the same curve. For example, the deviation between the two curves presented in Figure 4 corresponded to a difference in dead space of less than 3 ml.

DISCUSSION

Subjects 1 and 5 in Table II had dead spaces for helium which were significantly smaller than those for some of the other gases. However, for reasons beyond our knowledge or control, the variation among a series of dead space determina-

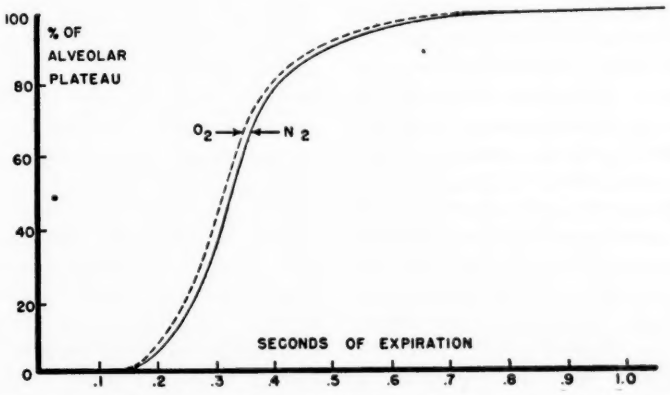

FIG. 4. SIMULTANEOUS RECORDING OF NITROGEN CONCENTRATION (NITROGEN METER) AND OXYGEN CONCENTRATION (MASS SPECTROMETER)

Both concentrations are expressed as percentages of their respective 'alveolar plateaus.' The difference in dead space volume resulting from the above shown difference in concentration is less than 3 ml.

tions on one occasion was not as great as the variation between the mean values of dead space determined on different occasions, in spite of the fact that the position of the subject, lung volume, inspired and alveolar gases, as well as the time of breathholding were apparently the same. For example, under controlled conditions, the carbon dioxide dead space of Subject 9 varied from 147 ml. to 186 ml. (unreported data; each the average of five observations) in one afternoon, a significant change. This type of variation occurred to some degree in all subjects. The small unavoidable differences in breathholding time which occurred were not great enough to have produced this variation nor was there a correlation. Therefore, we think that the physiological significance of these dead space differences is uncertain. The simultaneous use of the mass spectrometer and the nitrogen meter proved most useful when comparing the dead space for different gases because this unexplained variation between different breaths was eliminated. Unfortunately, helium could not be studied in this fashion, because it changes the sensitivity of the nitrogen meter. In any case the dead space for helium was not more than about 20 ml. smaller than the dead space for the other gases studied.

As stated above, Fowler (6) has shown that in the case of nitrogen in normal subjects by the time the rising concentration joins the 'alveolar plateau,' the expired breath contains pure alveolar gas. Since the O_2 and CO_2 concentrations in the expired breath, expressed as fractions of their extrapolated 'plateau' values, are the same as the N_2 concentration (Figure 4), we have concluded that the dead spaces for O_2 and CO_2 are also completely washed out by the time their respective concentration curves have joined their 'alveolar plateaus.'

Gas exchange in the dead space could be studied in a precise manner by means of the simultaneous nitrogen and oxygen (or carbon dioxide) records corrected to fractions of their respective alveolar concentrations. During the entire expiration the two curves coincided. There were two interesting exceptions to this generalization. Firstly, in some experiments there was a stepwise change in concentration at the foot of the S-shaped rise (Figure 5). Simultaneous records of nitrogen and oxygen (or carbon dioxide) concentrations in the expired gas demonstrated that the two gases followed the same concentration course during the step in the majority of instances. In those few experiments where this was not true, the nitrogen fraction was as often greater than the oxygen (or carbon dioxide) as *vice versa*. Therefore, we believe these steps did not represent significant gas exchange in the dead space. Secondly, in the simultaneous nitrogen and oxygen curves of all five subjects the oxygen concentration appeared to rise more rapidly than the nitrogen concentration (Figure 4), in particular after the breath was held for 20 seconds or more. This effect is equivalent to a time difference between the two concentration curves of 0.02 seconds, or to a decrease in O_2 dead space of about 2 ml. These differences were so small that we are unable to say at present whether they were artefacts or not.

Galdston and Horwitz (8) reported an average rise in carbon dioxide tension and fall in oxygen tension of 3.7 mm. Hg (about 0.52 per cent of an atmosphere change in carbon dioxide or oxygen concentration) in the supraglottic portion of the airway during periods of breathholding of up to 30 seconds. Forssander (9), who studied carbon dioxide only, showed that this exchange did not take place in the oral cavity. Following, as noted above, an inspiration of 100 per cent oxygen, simultaneous records of carbon dioxide and nitrogen concentrations in the expired gas showed that the carbon dioxide concentration did not rise (less than 0.05 per cent) until the nitrogen concentration started to rise, even after 30 seconds of breathholding. This made it unlikely that there was carbon dioxide exchange in that part of the expired gas that contained no alveolar gas (absolute dead space). The fact that the nitrogen and carbon dioxide concentration curves coincided during the rapid buildup of concentration in the expired gas made it improbable that a significant amount of carbon dioxide had diffused into the dead space. The oxygen concentration in the absolute dead space, under conditions comparable to those of Galdston, was difficult to interpret in terms of gas exchange because of the water vapor dilution mentioned above. As it was unlikely that oxygen would exchange to a greater extent than the far more soluble carbon dioxide, we do not believe that there was a significant exchange of carbon dioxide and oxygen between the dead space and the surrounding tissues.

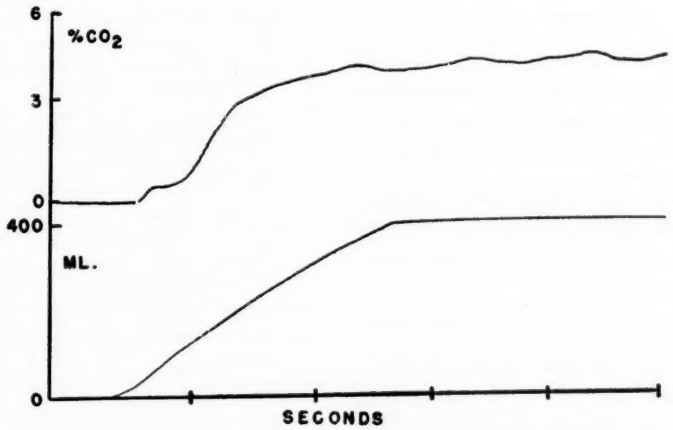

FIG. 5. REPRODUCTION OF A RECORD OF CARBON DIOXIDE CONCENTRATION AND VOLUME OF SUBJECT 5 DURING EXPIRATION

The co-ordinates are the same as in Figure 2. The upper line is the carbon dioxide concentration, and shows a 'step' at the foot of the S-curve, as well as cyclic concentration changes synchronous with the heart beat. The lower line is expired gas volume. The terminal horizontal part of this line is produced by the recording pen reaching its limit of movement.

Figure 5 also shows how the shape of a number of curves was apparently affected by the action of the heart. The small changes in concentration synchronous with the heart beat are much more prominent in the records of some subjects than in those of others, for no obvious reason. When present, they continue throughout each entire expiration. This 'cardiac ripple' was most prominent in oxygen and carbon dioxide curves but it was also present in a number of nitrogen and helium curves. We did not observe in any of our records of alveolar oxygen concentration the paradoxical inversion of the alveolar plateau late in expiration described by Cotton (10).

Fowler had shown that the nitrogen dead space decreased with increasing time of breathholding, and DuBois did the same for the carbon dioxide dead space. In Figure 3 we showed this decrease to be the same for oxygen, carbon dioxide and helium as for nitrogen. This decrease cannot be due mainly to diffusion because it is approximately the same for helium as for the other gases. The rapid diffusion of helium should have resulted in a smaller dead space. The most important cause of the decrease with time of the dead space volume is probably the churning action of the heart, as suggested by DuBois. Just why the curve is steepest for breathholding times up to 5 seconds is not clear. The shape of the curve suggests that at time zero the dead space might be at least twice as large as it is after 2½ seconds of breathholding. The curve further shows that the rate of decrease after 20 seconds of breathholding becomes very small indeed. This is in contradistinction to one of DuBois' subjects whose dead space was reduced to zero after 55 seconds of breathholding. None of our subjects approached zero values after 60 seconds of breathholding. Otherwise, our data for the dead space agree well with those of Fowler, DuBois, Pappenheimer, and Krogh to the extent that our studies were comparable with these.

The very first portion of every expired breath represents pure dead space gas without any admixture of alveolar gas. We found the volume of this 'absolute dead space' to vary considerably from breath to breath, but the simultaneous oxygen or carbon dioxide and nitrogen records showed that on the same breath it was the same for these three gases. We compared the absolute dead space of Subjects 1, 5, 6, 9, and 10 for helium and nitrogen, and found it to vary from 15 to 80 ml., but without any trend that would differentiate between these two gases.

SUMMARY

1. Using a rapidly recording mass spectrometer and a light weight spirometer with attached potentiometer, the respiratory dead space was estimated with oxygen, carbon dioxide, nitrogen and helium as indicator gases.

2. In five normal subjects ranging in age from 27 to 35 years, the dead space decreased to the same degree with increasing breathholding times for all four indicator gases.

3. The dead space for the four indicator gases was essentially the same in three of five subjects, and in the other two the dead space for helium appeared to be somewhat (20 ml.) smaller than for the other indicator gases. Breathing helium did not decrease the oxygen, carbon dioxide or nitrogen dead spaces.

4. Using the mass spectrometer simultaneously with a Lilly nitrogen meter, expiratory gas concentration curves were obtained in five subjects for oxygen, carbon dioxide, and nitrogen; these were so similar that there was no measurable difference in dead space volume.

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A COMPARISON OF INSULIN TREATMENT WITH AND WITHOUT ADDED CARBOHYDRATE IN HUMAN DIABETIC KETOSIS [1, 2]

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A controversy exists whether the administration of carbohydrates in addition to insulin in patients in diabetic acidosis actually speeds recovery from ketosis. The arguments used by the proponents (1–3) and opponents (4, 5) of the early use of carbohydrate are well known, and need not be elaborated. Franks, Berris, Kaplan, and Myers (5) found no difference in the average length of time it took for acetone to disappear from the urine by qualitative tests in diabetic acidosis whether early glucose was used or not. However, most investigators have found urinary ketones, particularly by qualitative tests, a poor guide to the degree of ketonemia. One reason for this is that urinary ketones continue to be excreted until blood levels reach extremely low levels (6), usually in sufficient quantities to cause a "4-plus" qualitative test.

To study the effect of carbohydrate on recovery from ketosis, the fall in total blood ketones in diabetic ketosis during insulin therapy, with and without the addition of carbohydrate, has been measured. The effectiveness of intravenous fructose in lowering blood ketone levels has been compared to glucose because of preliminary observations by Daughaday and Weichselbaum (7), since confirmed by others (8–10), that there was relatively normal utilization of fructose in diabetic acidosis.

METHODS

A direct comparison of therapies was made possible by inducing ketosis twenty times in eight diabetic patients. The subjects (five women and three men) ranged in age from nineteen to sixty-seven years and were free of clinically demonstrable cardiac and renal disease or acute infections. Insulin was withdrawn from one to three days, and an attempt was made to achieve equivalent initial degrees of ketosis in all studies on the same patient. The initial level of blood ketones averaged 5.1 mM per L. In spite of marked polyuria all patients remained hydrated; slight vomiting occurred in two patients; and in only one instance did Kussmaul respirations develop. Regular insulin was given subcutaneously in doses adequate to control hyperglycemia when saline was given; and insulin dosage was constant for all studies on the same patient. Ten per cent glucose or fructose in water was administered intravenously at a rate of 0.8 Gm. per Kg. per hour for four hours. Constant infusion rates were maintained by continuous observation. In control experiments, the patients received insulin and comparable volumes of normal saline. Different sequences of therapeutic regimens were used in individual patients. All infusions were given early in the morning in the fasting state, usually fourteen hours after the previous meal. Blood levels and urinary excretion of glucose, fructose, and total ketones were measured hourly for six hours. Total blood and urine ketones (including acetone, acetoacetic acid, and betahydroxybutyric acid) were measured by the method of Weichselbaum and Somogyi (11) and calculated in terms of millimoles per liter. Blood and urine total reducing sugars were determined in duplicate by Nelson's method (12). Fructose determinations were performed in duplicate by the method of Weichselbaum, Margraf, and Elman (13). Glucose values were obtained by subtracting fructose concentration from the concentration of total reducing sugars. The results were analyzed statistically by standard methods (14, 15).

RESULTS

The blood and urine glucose and fructose values during the therapeutic procedures for each patient are presented in Table I. The average blood sugars in the six patients who received both saline and insulin are shown in Figure 1A. An average dose of forty-five units of insulin was sufficient to lower the blood sugar to normal levels. After fructose and insulin there was an initial rise in the total blood sugar somewhat greater in the glu-

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TABLE I

Individual blood and urine sugars

Patient Age-Sex Weight Previous insulin dose	Therapy sugar given I.V. *0.8 Gm./Kg./Hr.*	Blood sugar *Mg. %*	0	1	2	3	4	5	6	Glycosuria *Gm./6 Hr.*	Sugar given *Gm.*	Gm. retained	Per cent of infused sugar retained %
C. J.		Insulin dose	25u	25u	25u								
41 F	*I+S	Glucose	324	260	168	100	55	82	60	15			
50 Kg.	F+I	Glucose	312	422	344	300	281	152	80	43			
35u PZI		Fructose	0	49	71	60	72	11	0	13	160	104	65
	G+I	Glucose	443	568	435	646	566	296	200	101	160	59	37
E. S.		Insulin dose	25u	15u	15u								
22 M	I+S	Glucose	410	345	223	176	150	99	50	38			
41.4 Kg.	F+I	Glucose	408	420	381	318	236	174	134	59			
35u Globin		Fructose	0	30	22	27	36	6	0	9	132	64	49
	G+I	Glucose	370	480	475	465	373	232	162	84	132	48	36
M. H.		Insulin dose	25u	15u									
67 F	I+S	Glucose	990	990	784	668	500	347	278	71			
55 Kg.	F+I	Glucose	700	941	894	769	609	546	477	67			
90u 2:1 mix		Fructose	0	79	56	61	81	38	21	7	176	102	58
W. G.		Insulin dose	25u										
42 F	I+S	Glucose	495	555	407	338	268	247	241	20			
60 Kg.	F+I	Glucose	530	565	550	547	509	387	422	89			
100u		Fructose	0	30	40	43	51	10	0	12	192	91	47
2:1 Mix	G+I	Glucose	550	677	733	687	612	545	491	149	192	43	22
J. S.		Insulin dose	25u	15u	10u								
20 M	I+S	Glucose	595	519	375	293	154	99	44	24			
60 Kg.	F+I	Glucose	648	586	563	473	(501)†	353	260	50			
40u Globin		Fructose	0	15	72	38	(57)†	2	0	2	192	140	73
V. H.		Insulin dose	25u										
19 F	I+S	Glucose	590	459	288	237	157	109	91	30			
38.6 Kg.	F+I	Glucose	386	478	478	441	374	263	178	42			
15u PZI		Fructose	0	50	50	65	74	6	0	9	124	73	62
E. H.		Insulin dose	40u										
20 M	F+I	Glucose	258	247	267	269	211	171	150	26			
58.6 Kg.		Fructose	0	51	58	39	48	12	0	11	188	151	80
40u PZI	G+I	Glucose	263	443	479	460	418	318	270	125	188	63	33
V. B.		Insulin dose	40u	10u									
23 F	F+I	Glucose	393	324	290	238	216	174	104	43			
50 Kg.		Fructose	0	56	80	80	59	4	0	10	160	107	67
20u Globin 15u Reg	G+I	Glucose	350	484	441	437	435	200	154	78	160	82	51

* I = Insulin, F = Fructose, G = Glucose.
† These are missing data calculated by the method of Snedecor (14) and used for statistical purposes only.

cose than in the fructose fraction. The total blood sugar then rapidly decreased, paralleling the fall after insulin alone. Blood fructose levels during infusion averaged 51 mg. per cent, then fell, being measurable in only one case after the first post-infusion hour. With the comparison of glucose and insulin to fructose and insulin in five patients (Figure 1B) the average insulin dose was 52 units. Glucose produced a more sustained rise in the blood sugar than did fructose during the infusion, after which the blood sugar rapidly fell.

The comparative sugar balance during the six hour experimental period is charted in Figure 2. When insulin and saline were given (Figure 2A) there was an average glycosuria of 33 grams. Fructose and insulin treatment was followed by a total glycosuria averaging 67 grams, of which only nine grams were fructose. Ninety-six grams of the infused fructose were retained. In the comparison of the two sugars (Figure 2B); fructose and insulin produced less total glycosuria and greater sugar retention than glucose and insulin.

Blood and urine ketone levels during the therapeutic procedures are given in Table II. The average ketone values for the therapeutic procedures for each hourly period are plotted in Figure 3 and the calculated slopes indicated. The rate of fall of blood ketones was significantly greater after fructose and insulin than after saline and insulin in five of the six patients compared, and was highly significant for the average blood ketone level for all six patients (Figure 2A). In the exception, J. S., the severity of ketosis during fructose and insulin therapy was obviously greater than in any of the other experiments, since he had Kussmaul respirations. One might argue that the higher initial ketone levels in the patients receiving fructose and insulin had a bearing on the greater rate of fall of ketones after fructose and insulin. However, the experience of Martin and Wick (6) in a large series of patients corresponds with our experience that the greater the initial ketone level in any one patient, the more difficult it is to suppress ketosis by any form of therapy.

The slightly more rapid fall of ketones produced by fructose and insulin as compared with glucose and insulin was not significant (P greater than .2) for any of the individual pair comparisons, or for the average blood ketone values (Figure 3B). In the three subjects studied the average blood ketone level fell significantly faster (P less than .05) during therapy with glucose and insulin than with insulin alone. In general, urinary ketones tended to decrease as blood ketones fell, but there was a poor correlation between blood and urine ketone levels. There was no significant difference between the average total ketonuria following any of the therapeutic procedures.

The effect of insulin administration on the assimilation and antiketogenic action of fructose was

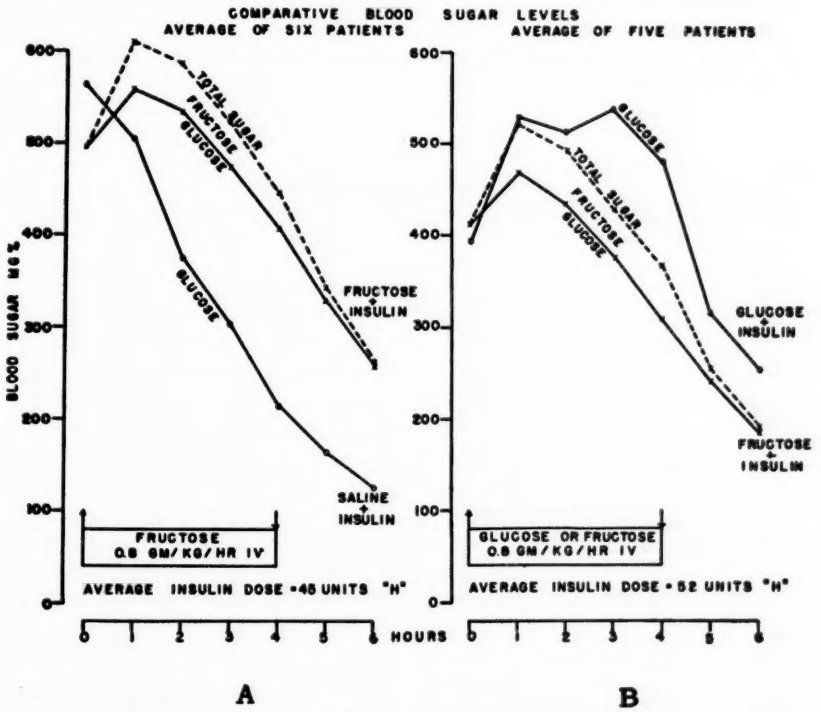

FIG. 1. A. COMPARISON OF THE AVERAGE BLOOD SUGARS DURING THERAPY WITH FRUCTOSE—INSULIN AND SALINE—INSULIN IN THE SAME SIX PATIENTS. B. DURING THERAPY WITH GLUCOSE—INSULIN AND FRUCTOSE—INSULIN IN THE SAME FIVE PATIENTS

Blood fructose concentrations during fructose therapy are represented by the distance between the total sugar (dotted line) and blood glucose (solid line).

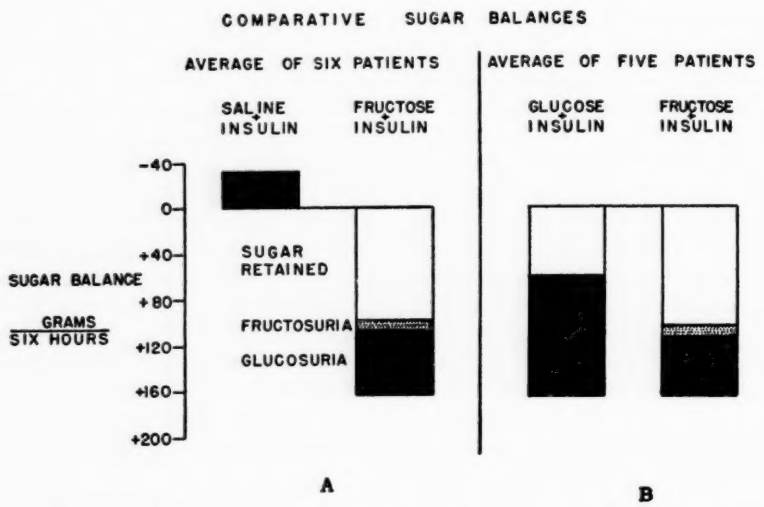

FIG. 2. A. COMPARISON OF THE AVERAGE SUGAR BALANCE AND GLYCOSURIA DURING THERAPY WITH SALINE—INSULIN AND FRUCTOSE—INSULIN IN THE SAME SIX PATIENTS. B. DURING THERAPY WITH GLUCOSE—INSULIN AND FRUCTOSE—INSULIN IN THE SAME FIVE PATIENTS

During sugar infusions the total amount infused is equal to the sugar retained plus the total glycosuria.

TABLE II

Individual blood and urine ketones

Subject	Therapy	Blood ketones mMoles/Liter *Hours* 0	1	2	3	4	5	6	Slope	Urine ketones mMoles 6 hours	Probability for slopes
C. J.	I+S*	3.0	2.6	3.0	2.1	1.7	.9	(1.4)†	− .341	9.7	IS vs FI = <.01
	F+I	5.9	4.3	3.0	1.4	.8	.5	.4	− .954	16.9	FI vs GI = >.2
	G+I	4.4	4.2	3.2	1.9	1.2	.4	.4	− .717	13.8	IS vs GI = <.1
E. S.	I+S	3.9	3.7	2.9	2.7	2.7	1.5	1.4	− .430	13.7	IS vs FI = <.01
	F+I	5.9	4.8	5.1	3.6	2.0	1.5	.8	− .893	15.0	FI vs GI = >.2
	G+I	6.2	2.6	2.3	3.7	2.2	.6	.4	− .767	15.9	IS vs GI = <.05
M. H.	I+S	6.5	5.2	5.6	5.4	5.8	7.3	5.2	+ .011	57.2	IS vs FI = <.05
	F+I	6.7	7.9	6.7	5.3	6.1	3.8	3.1	− .704	37.3	
W. G.	I+S	3.0	3.5	4.7	2.9	2.5	3.0	4.0	− .010	18.1	IS vs FI = >.2
	F+I	3.0	2.5	2.0	2.0	1.6	1.8	2.0	− .173	12.2	FI vs GI = >.2
	G+I	4.9	4.5	4.1	4.3	4.0	3.8	4.6	− .086	52.2	IS vs GI = >.2
J. S.	I+S	6.5	6.7	5.9	5.3	4.0	4.0	4.0	− .510	58.0	IS vs FI = >.2
	F+I	7.8	8.9	9.9	6.0	(6.5)†	6.4	7.4	− .349	81.6	
V. H.	I+S	3.6	3.4	2.6	2.8	2.8	2.2	2.2	− .229	14.6	IS vs FI = <.05
	F+I	8.5	7.3	5.4	3.3	2.1	1.8	1.3	−1.245	14.5	
E. H.	F+I	2.8	2.6	1.9	1.5	.4	.4	.02	− .506	5.7	FI vs GI = >.2
	G+I	2.3	3.6	2.3	1.4	1.1	.7	.6	− .414	3.8	
V. B.	F+I	5.0	5.1	2.1	1.5	1.0	.8	.8	− .856	26.9	FI vs GI = >.2
	G+I	4.1	3.6	2.5	1.1	.5	.2	.3	− .726	18.7	

* I = Insulin, F = Fructose, G = Glucose.
† These are missing data calculated by the method of Snedecor (14) and used for statistical purposes only.

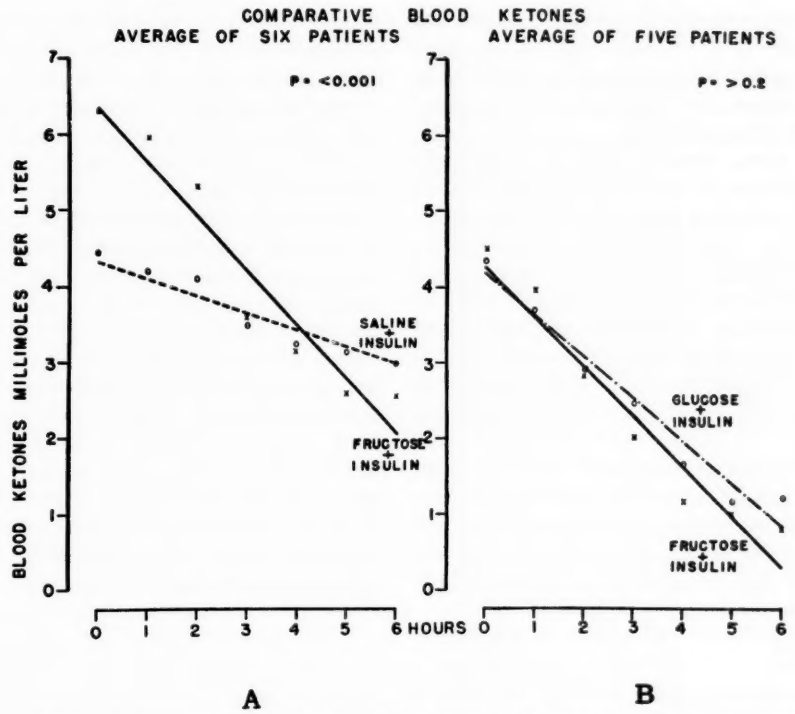

FIG. 3. A. COMPARISON OF THE SLOPES OF THE AVERAGE HOURLY BLOOD KETONES DURING THERAPY WITH SALINE—INSULIN AND FRUCTOSE—INSULIN IN THE SAME SIX PATIENTS. B. DURING THERAPY WITH GLUCOSE—INSULIN AND FRUCTOSE—INSULIN IN THE SAME FIVE PATIENTS

P. = Probability.

studied in one patient. In the presence of insulin (Figure 4A) the blood sugar curves followed the typical pattern. Blood ketones fell steadily from 5.9 to 0.8 mM per L. When fructose was given without insulin (Figure 4B) the total blood sugar continued to rise during the infusion due to an increase in blood glucose. Blood fructose levels were no higher without insulin than when insulin was given. There was an initial rapid fall of ketones to half the initial level, but there was no further fall during the experimental period. Total glycosuria during fructose and insulin was 68 grams and this increased to 103 grams during fructose without insulin due to an increase in the glucose fraction.

DISCUSSION

Three processes should be considered in explaining the more rapid drop in blood ketones observed when carbohydrate was given: One, increased renal loss of ketones; two, increased peripheral utilization of ketones; and three, decreased hepatic production of ketones. The first possibility can be eliminated because in our experiments there was no significant difference in the total ketonuria induced by any of the therapeutic procedures.

The second possibility would be that the administered carbohydrate might promote increased peripheral utilization of ketones. Evidence against such a mechanism has been offered by Chaikoff and Soskin (16) who found no appreciable difference between the rates of ketone utilization in the peripheral tissues of the normal and diabetic hepatectomized dog. This finding has been confirmed by others using various techniques as reviewed by Stadie (17). Insulin (16), and glucose administration (18) have no effect on the peripheral utilization of ketones as determined in

the eviscerated animal. However, Somogyi and Weichselbaum (19) postulate that carbohydrates cause a suppression of extrahepatic ketone utilization to account for temporary rises in blood ketones after glucose feeding.

It would seem, therefore, that the third possibility, an inhibition of hepatic ketogenesis, is more important in speeding recovery from diabetic ketosis (18, 19). Non-availability of carbohydrate metabolites, possibly at the level of incorporation into the Krebs cycle (20) is an important factor in ketone production. Weinhouse, Millington, and Friedman (21) found that acetoacetate production from C^{14} labeled fatty acids in liver slices was decreased by addition of glucose to the incubation medium.

The importance of insulin in inhibiting ketogenesis in diabetic subjects is well known clinically and has been confirmed in experimental animals (17). Stadie, Zapp, and Lukens have reported (22) that the addition of insulin to liver slices from diabetic cats incubated in a carbohydrate free medium was ineffective in suppressing ketone production. When fructose was added to the medium, inhibition of ketogenesis was maximal only in the presence of insulin. Under certain circumstances insulin may actually increase ketone production (23, 24).

The more rapid recovery from ketosis which we have observed following carbohydrate administration may be attributed to the increased utilization of sugar observed in these patients. It is felt that the sodium chloride received for osmotic reasons during the control but not during carbohydrate administration experiments was without significant effect on ketosis. As indicated in Figure 2, a negative carbohydrate balance is converted into a positive one by administration of carbohydrate. An estimate of the total carbohydrate taken up by the tissues has been made under the varied conditions studied. The extracellular glucose was estimated on the basis of the results of Wick,

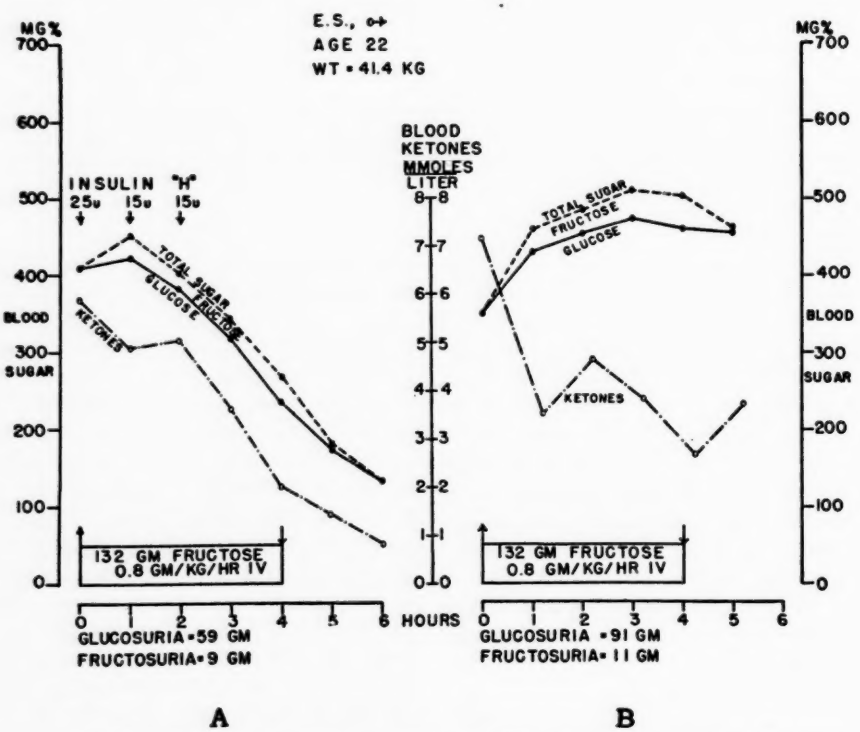

FIG. 4. A. THE EFFECT OF INSULIN ON THE BLOOD SUGARS AND KETONES DURING FRUCTOSE THERAPY. B. BLOOD SUGARS AND KETONES DURING FRUCTOSE THERAPY ALONE

Drury, and MacKay (25) indicating a glucose space of 30 per cent body weight. From the urinary excretion of sugar, the grams administered, and the extracellular sugar at the end of the experimental period a figure of body carbohydrate uptake can be obtained.[4] The average carbohydrate uptake by the tissues with insulin and saline was 34 grams, with glucose and insulin 81 grams, and with fructose and insulin 127 grams. As noted previously we found no difference in the antiketogenic effect of intravenous glucose or fructose in the presence of insulin. This may be due to the fact that, under the conditions of our experiments, the carbohydrate utilization with glucose administration was sufficient for maximum antiketogenic effect.

Despite the fact that fructose is removed from the blood of diabetic subjects (27, 28) at virtually a normal rate without insulin, insulin is necessary for the full antiketogenic effect. The infusion of fructose to one patient without insulin resulted in a calculated removal of only 14 grams of sugar by the tissues. The same patient receiving fructose and insulin had a calculated tissue uptake of 98 grams. The more effective suppression of ketogenesis can be attributed to the increased tissue carbohydrate uptake. The decreased uptake of carbohydrate in the absence of insulin is not a failure to remove fructose from the blood. It is due to the conversion of fructose to glucose by the liver (29) and perhaps intestines (30), and subsequent release of glucose from the liver cells to the blood. The mechanism of this conversion has been described by Cori, Ochoa, Slein, and Cori (29).

One disadvantage of early glucose therapy in the diabetic has been the greater hyperglycemia with its resultant intracellular dehydration, osmotic diuresis, and loss of electrolytes. As shown in these experiments fructose judiciously used can minimize these disadvantages. This work supports the argument that carbohydrate, in addition to adequate insulin and fluid, speeds recovery from diabetic ketosis.

[4] The calculations were performed according to the following example for the average of six patients receiving saline and insulin. Glucose space = .30 × 50.8 (average weight in Kg.) = 15.2 liters. Total available sugar = − 33 Gm. (sugar balance from Figure 2) + [576 (mg./100 cc.) (initial blood sugar) × 15.2 liters] = 53 Gm. Sugar left in glucose space at the end of the six hour experimental period = 127 (mg./100 cc.) (final blood sugar) × 15.2 liters = 19 Gm. Sugar utilized = 53 − 19 = 34 Gm. Not considered in this and subsequent calculations were approximately 25 grams of sugar put out by the liver in the first hour after insulin administration, and later taken up by the tissues, as determined in comparable patients by Bondy, Bloom, Whitner, and Farrar (26).

SUMMARY

1. The fall in the total blood ketones in diabetic acidosis during insulin therapy with and without the addition of carbohydrate has been measured. A direct comparison of therapies was accomplished by inducing ketosis 20 times in eight diabetic patients.

2. The blood ketone levels fall more rapidly following intravenous glucose and fructose than with saline in insulin treated patients with diabetic ketosis. No significant difference in the rate of fall of blood ketones was found between glucose and fructose.

3. When insulin was omitted, intravenous fructose was less effective in lowering blood ketones in one patient.

4. Hyperglycemia was less prolonged and glycosuria was smaller after intravenous fructose than glucose in insulin treated patients with diabetic ketosis.

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THE BLOOD FLOW AND OXYGEN CONSUMPTION OF THE BRAIN IN PATIENTS WITH ESSENTIAL HYPERTENSION BEFORE AND AFTER ADRENALECTOMY [1]

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It has been shown that cerebral oxygen consumption and blood flow are normal and cerebral vascular resistance is increased in patients with essential hypertension (1). Clinicians have questioned the advisability of lowering arterial pressures in hypertensive patients because of the possible untoward effects consequent to decreased cerebral blood flow (2, 3). We have studied the effect of hypotension, induced by various means, on the oxygen consumption and blood flow of the brain.

During the hypotension obtained one hour after the injection of dihydroergocornine (4), protoveratrine (5), hexamethonium (6), and l-hydrazinophthalazine (7), cerebral blood flow and oxygen uptake were unchanged. Cerebral oxygen uptake was unchanged and cerebral vascular resistance was decreased during the hypotension induced by differential spinal sympathetic block (8). After thoracolumbar sympathectomy, cerebral blood flow and oxygen uptake remained constant and the cerebral vascular resistance was reduced toward normal values three weeks to six months after operation (9). Thus, in most of these instances, cerebral blood flow was not reduced and cerebral oxygen consumption remained unchanged when the arterial pressure was lowered either by the drugs tested or by surgical sympathectomy. These hemodynamic observations suggested that the cerebral circulation remains adequate when the blood pressure is lowered. The increased cerebral vascular resistance in hypertension apparently can be reduced by certain measures without reducing cerebral oxygen consumption and cerebral blood flow.

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The present investigation was initiated to explore the effect of another hypotensive procedure, subtotal adrenalectomy (approximately 90 per cent), on arterial and jugular venous blood constituents, cerebral oxygen uptake, cerebral blood flow and cerebral vascular resistance in patients with severe hypertension. The tests were made at approximately the same postoperative intervals as in the postsympathectomy study (9).

METHODS

1. Choice of subjects: This report is based upon the data obtained from seven patients. In all patients cerebral blood flow studies were made before and again 1 to 15 months after subtotal adrenalectomy. Four patients (I. B., A. F., J. D., E. D.) had subdiaphragmatic sympathectomy at the time of the subtotal adrenalectomy (95 to 98 per cent) and were studied three to eight months postoperatively. Two patients (S. W. and T. C.) had subtotal adrenalectomy (91 to 93 per cent) alone. However, the blood pressure response of one (T. C.) was unsatisfactory and he later submitted to subdiaphragmatic sympathectomy. One patient (S. C.) had a thoracolumbar sympathectomy (Smithwick) and unilateral adrenalectomy nine months before 90 per cent resection of the remaining adrenal was accomplished.

Studies were repeated in three patients (I .B., A. F., and S. W.) approximately one year after operation. The patients were evaluated preoperatively and grouped in the life expectancy classification of Smithwick (10), patients in group IV having the poorest prognosis.

2. Adrenal cortical substitution therapy: Five of the seven patients tested were receiving cortisone at the time of their initial postadrenalectomy cerebral blood flow study. These patients were maintained with oral cortisone (6 to 25 mg. per day) alone or cortisone and buccal desoxycorticosterone.[2] T. C. (along with S. C.) required no cortisone at the time of the initial postadrenalectomy study, but he required cortisone and desoxycorti-

[2] Generous supplies of adrenal cortical extract, desoxycorticosterone acetate and cortisone were made available for our studies by the Upjohn, Ciba, Schering and Merck Companies.

costerone at the time of the postsympathectomy study, presumably because of interference with adrenal cortical remnants during the sympathectomy operations. I. B. did not require cortisone at the time of the second postadrenalectomy study. All patients were ambulatory and outpatients at the time of the postadrenalectomy tests.

3. Technique of study: Cerebral blood flow was determined in the supine position by the nitrous oxide method and cerebral oxygen consumption, mean arterial pressure and arterial and jugular venous blood constituents were determined as previously described (1, 7).

RESULTS

Table I contains the results obtained in each test. The means of eleven[3] observations in the seven patients after adrenalectomy are compared with the mean preoperative values in Table II. Mean preoperative values in this group of patients were not significantly different from those observed in a larger group of hypertensive patients (11). After adrenalectomy, mean values for cerebral oxygen consumption, jugular venous oxygen content, jugular venous oxygen saturation and tension, arterial and jugular venous carbon dioxide tension and cerebral respiratory quotient were not appreciably altered. The averages showed a slight increase in cerebral blood flow and a reduction in cerebral arteriovenous oxygen difference. The reductions in mean arterial pressure, cerebral vascular resistance, arterial and jugular hydrogen ion concentration, arterial and jugular venous carbon dioxide content and arterial oxygen content were also significant ($p < 0.05$).

[3] The postsympathectomy-unilateral adrenalectomy results in patient S. C. shown in Table I are not included. Both postadrenalectomy results in patient T. C. are included.

DISCUSSION

Wilkins' hemodynamic studies during procedures designed to lower the blood pressure of hypertensive patients have revealed that total and regional blood flows remained adequate in spite of sizeable lowerings of the pressure (12). The observations of Wilkins and his co-workers, the present study, and previously reported studies of cerebral blood flow and oxygen consumption in hypertensive patients (4–9) are reassuring in the face of the argument that hypertension is merely an adjustment to organic vascular disease and that to disturb this adjustment will cause cerebral or other regional circulatory embarrassment.

The data obtained by this investigation indicated, in the hypertensive patients studied, that the mean cerebral oxygen consumption, blood flow and arteriovenous oxygen difference and jugular venous oxygen tension were within the normal range and

TABLE I

Effects of subtotal adrenalectomy combined with sympathectomy on cerebral circulation and oxygen metabolism

Patient	Sex	Age	Smith-wick group	Carbon dioxide								Blood pH. 37°C.			
				Content *Volumes %*				Tension				Arterial		Venous	
				Arterial		Venous		Arterial		Venous					
				B*	A†	B	A	B	A	B	A	B	A	B	A
I. B.	F	44	IV	50.5	40.5 42.2	58.4	47.2 48.9	39	31 37	53	40 44	7.45	7.44 7.37	7.35	7.38 7.32
A. F.	F	50	IV	56.2	42.6 51.0	62.0	48.9 56.3	47	40 40	54	52 55	7.40	7.35 7.35	7.35	7.26 7.29
J. D.	M	42	II	47.8	41.5	56.4	49.9	40	37	50	46	7.41	7.40	7.36	7.36
E. D.	M	43	II	48.1	47.3	53.5	52.2	39	42	48	52	7.43	7.38	7.36	7.31
S. W.	M	47	IV	50.4	49.0 32.5	57.5	54.8 38.6	41	38 38	50	46 45	7.41	7.42 7.26	7.37	7.38 7.20
T. C.	M	42	IV	59.1	45.3 44.7	66.9	51.8 52.8	47	36 46	58	47 58	7.45	7.44 7.31	7.40	7.37 7.26
S. C.	F	33	IV	45.1	46.8 37.5	52.4	55.3 43.2	30	38 38	42	52 47	7.50	7.41 7.30	7.40	7.33 7.26

* B denotes study made before surgical procedure.
† A denotes study made after surgical procedure.

TABLE I—*Continued*

Patient	Sex	Age	Smithwick group	Oxygen								Cerebral									
				Content *Volumes %*				Tension *mm. Hg*		Saturation *% oxy. Hb.*		Blood flow‡		Oxygen uptake‡		Vascular resistance§		Respiratory quotient		Mean art. press. *mm. Hg*	
				Arterial		Venous		Venous		Venous											
				B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
I. B.	F	44	IV	17.1	14.9 15.5	9.1	8.2 7.9	30	27 30	55	51 52	50	44 44	4.0	2.9 3.3	3.7	2.7 2.6	0.99	1.00 0.88	186	119 116
A. F.	F	50	IV	15.2	13.6 14.8	8.7	7.3 9.0	32	29 35	58	47 61	52	64 64	3.4	4.0 3.7	3.4	1.5 1.8	0.89	1.00 0.91	175	98 116
J. D.	M	42	II	19.5	18.9	10.7	10.7	30	26	55	47	38	37	3.3	3.0	4.6	3.9	0.98	1.02	174	143
E. D.	M	43	II	18.5	18.0	12.2	12.9	39	39	69	68	72	86	4.5	4.4	2.2	1.4	0.86	0.96	158	116
S. W.	M	47	IV	17.2	13.8 17.3	9.8	7.8 10.4	30	32 37	55	58 61	51	56 49	3.8	3.4 3.4	2.8	2.3 2.4	0.96	0.97 0.88	144	126 116
T. C.	M	42	IV	19.5	19.7 19.4	11.5	12.5 11.3	30	36 36	57	65 61	49	63 72	3.9	4.5 5.8	3.7	2.2 1.5	0.98	0.90 1.00	181	138 107
S. C.	F	33	IV	14.8	17.1 14.4	7.6	8.5 8.6	24	29 29	44	51 47	55	39 74	4.0	3.4 4.3	3.2	4.3 1.4	1.01	0.99 0.98	175	168 100

‡ cc./100 Gm./min.
§ Cerebral vascular resistance is mean arterial pressure divided by cerebral blood flow expressed as mm. Hg/cc. blood/100 Grams brain/minute.

TABLE I—*Continued*

Patient	Sex	Age	Smithwick	Time after operation *Months*	Type of surgical procedure
I. B.	F	44	IV	3 12	98% adrenalectomy and bilateral subdiaphragmatic sympathectomy and splanchnicectomy.
A. F.	F	50	IV	3 12	98% adrenalectomy and bilateral subdiaphragmatic sympathectomy and splanchnicectomy.
J. D.	M	42	II	8	98% adrenalectomy and bilateral subdiaphragmatic sympathectomy and splanchnicectomy.
E. D.	M	43	II	3	95% adrenalectomy and bilateral subdiaphragmatic sympathectomy and splanchnicectomy.
S. W.	M	47	IV	1 15	93% adrenalectomy.
T. C.	M	42	IV	3 6	91% adrenalectomy. Bilateral subdiaphragmatic sympathectomy and splanchnicectomy.
S. C.	F	33	IV	3 14	Smithwick sympathectomy and total right adrenalectomy. 90% left adrenalectomy, *i.e.*, 95% total adrenalectomy.

were not reduced by the 30 per cent decrease in mean arterial pressure after adrenalectomy.

When the mean reductions in cerebral vascular resistance and mean arterial pressure after sympathectomy (9) were compared with the reductions observed in the patients who had sympathectomy plus adrenalectomy (eight observations in six patients), it is apparent that the reductions observed after adrenalectomy combined with sympathectomy were significantly greater ($p < 0.01$) than after sympathectomy alone (Table III).

The greater reduction in arterial carbon dioxide content and arterial pH after adrenalectomy (Tables II and III) are unexplained. The changes in arterial carbon dioxide content, tension and pH in individual patients did not correlate well with the increased cerebral blood flow, reduced cerebral vascular resistance or reduced mean arterial pressure. All but one patient (J. D.) have shown an increase in arterial hydrogen ion concentration when measurements were made three to fifteen months after adrenalectomy. This patient had the least reduction (18 per cent) in mean arterial pressure of this group.

The whole blood buffer base, (BB +) b, is the quantity that gives a true measure of acid or alkali deficit or excess in the extracellular fluid. When the mean values of Table II were entered on

TABLE II

*Mean changes in cerebral hemodynamics and oxygen metabolism and blood constituents of patients with essential hypertension after adrenalectomy**

	B†	A†	s.d.†	t†	p†
Mean arterial pressure	170	118	23.6	7.3	<0.01
Cerebral blood flow	52	59	10.2	2.2	0.05
Cerebral vascular resistance	3.4	2.2	0.6	6.0	<0.01
Cerebral oxygen uptake	3.8	3.9			
Cerebral arteriovenous oxygen difference	7.5	6.7	0.5	5.3	<0.01
Jugular venous oxygen content	9.9	9.7			
Jugular oxygen saturation	56	56			
Jugular pH	7.37	7.32	0.06	2.8	<0.05
Jugular oxygen tension	31	32			
Femoral arterial oxygen content	17.4	16.4	1.1	3.0	<0.05
Arterial carbon dioxide content	51.0	43.1	5.3	5.0	<0.01
Arterial carbon dioxide tension	40	38			
Jugular venous carbon dioxide content	58.1	49.5	5.5	5.2	<0.01
Jugular venous carbon dioxide tension	51	48			
Arterial pH	7.44	7.37	0.06	3.7	<0.01
Cerebral respiratory quotient	0.95	0.95			

* The averages include all eleven post adrenalectomy observations (1 month to 15 months) in seven patients regardless of time after operation.

† B denotes values preoperatively; A values postoperatively; s.d. denotes standard deviation of individual differences; t the ratio of difference between means and estimated standard deviation; and p, probability. Units are same as in Table I.

the nomogram of Singer and Hastings (13), the (BB +) b before adrenalectomy was within the normal range, 50 mEq. per L. and after adrenalectomy was below normal, being 42 mEq. per L. This suggested a metabolic acidosis with the base deficit probably caused by loss of sodium.

As is well known adrenal cortical insufficiency is associated with a loss of sodium, chloride, and bicarbonate and retention of potassium (14). It has been stated that the loss of sodium exceeds that of chloride and in addition urinary ammonia excretion is decreased (15). These factors would combine to produce a metabolic acidosis. While no measurements of excretion were made in our patients, mild adrenal cortical insufficiency cannot be excluded.

TABLE III

*Mean changes and standard deviation of individual differences in cerebral hemodynamics and oxygen metabolism of patients with essential hypertension after sympathectomy combined with adrenalectomy compared with the changes observed after sympathectomy**

Operative procedure and number of observations	Mean arterial pressure	Cerebral vascular resistance pressure/flow	Cerebral blood flow	Cerebral arterio-venous oxygen differences	Jugular oxygen content	Arterial carbon dioxide content
	mm. Hg		*cc./100 Gm./min.*	*vols. %*	*vols. %*	*vols. %*
Adrenalectomy combined with sympathectomy (8 observations in 6 patients)	−61 ± 17†	−1.4 ± 0.6	8 ± 11	−0.8 ± 0.6	−0.5 ± 0.9	−7.7 ± 4.5
Sympathectomy (9 observations in 9 patients—Data of Shenkin)	−20 ± 23	−0.5 ± 0.5	2 ± 11	−0.3 ± 1.0	−1.4 ± 1.0	−0.2 ± 4.0
Statistical significance of difference means	$p < 0.01$	$p < 0.01$	$p > 0.1$	$p > 0.1$	$p > 0.05$	$p < 0.01$

* After sympathoadrenal surgery greater reductions (significant difference of difference means) were observed in mean arterial pressure, cerebral vascular resistance and arterial carbon dioxide content.

† Denotes the mean change with the direction denoted by plus or minus and the mean changes are followed by the standard deviation of the individual differences.

Our data on this metabolic acidosis after adrenalectomy showing an increased blood flow to the brain are at variance with the experimentally induced acidosis reported by Schieve and Wilson (16). These investigators observed a reduction in cerebral blood flow and an increase in cerebral arteriovenous oxygen difference and cerebral vascular resistance. The metabolic acidosis of diabetes which Kety, Polis, Nadler, and Schmidt studied (17) also showed a reduced cerebral blood flow and increased cerebral vascular resistance. However, cerebral arteriovenous oxygen difference did not change with the restoration to normal of both the arterial hydrogen ion concentration and arterial carbon dioxide tension. The mean cerebral oxygen uptake of the diabetic patients was increased toward normal as the acidosis was corrected. Thus, the results observed in diabetic acidosis are probably not pertinent as mean cerebral oxygen consumption was unchanged in the acute experiments of Schieve and Wilson as well as in the postadrenalectomy studies.

The data presented afford no direct evidence bearing upon the mechanism by which the cerebral vascular resistance was reduced following sympathoadrenal surgery. On the other hand the hypothesis (9) that an intrinsic mechanism reciprocally adjusts the cerebral vascular resistance to alterations of perfusion pressure does not appear to be invalidated by these studies. We have shown that cerebral blood flow is increased not only with excess hydrogen ions but is also increased when 5 per cent carbon dioxide is inhaled (18) so that chemical factors as well as nervous must be considered.

The observations of Clark, Crosley, and Barker on renal blood flow, renal vascular resistance and glomerular filtration rate in patients studied before and after subtotal adrenalectomy are of interest (19). The change in glomerular filtration rate after adrenalectomy was found to be small and variable. In five of six patients effective renal blood flow increased at a time when mean arterial blood pressure was decreased by adrenalectomy, thus indicating a reduction in overall renal vascular resistance.

SUMMARY

Studies are reported of cerebral blood flow and oxygen consumption and arterial and internal jugular blood gases in seven patients with severe essential hypertension before and after a reduction in blood pressure achieved by 90 per cent adrenalectomy alone or combined with sympathectomy.

Mean values of oxygen consumption, jugular venous oxygen content, and jugular venous oxygen tension remained essentially unchanged and cerebral blood flow increased slightly after adrenalectomy.

The high cerebral vascular resistance of essential hypertension has been lowered toward the normal range. The mechanism of the reduction has not been established. A compensated metabolic acidosis was observed after subtotal adrenalectomy. The mean reduction in cerebral vascular resistance as well as the mean decrease in mean arterial pressure appeared to be greater after combined sympathectomy-adrenalectomy than the reductions observed after sympathectomy alone.

Data from this and other studies of the effect of reduction of blood pressure by surgical procedures and depressor drugs suggest that the increased cerebral vascular tone of certain selected hypertensive patients is reversible, and that cerebral oxygen uptake and jugular venous oxygen tension remain constant in the supine position when the perfusion pressure is lowered by these procedures.

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CEREBRAL OXYGEN CONSUMPTION IN ESSENTIAL HYPERTENSION [1]

CONSTANCY WITH AGE, SEVERITY OF THE DISEASE, SEX, AND VARIATIONS OF BLOOD CONSTITUENTS, AS OBSERVED IN 101 PATIENTS

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It has been shown that patients with essential hypertension have the same cerebral oxygen demands as normotensive subjects (1–3). Thirty-four observations in 14 normotensive subjects formed the basis of Kety and Schmidt's report (1). Recently, measurements in six patients with essential hypertension have been reported (2), as part of a study of the effects of aging, arteriosclerosis and hypertension upon the cerebral circulation.

Our initial report included studies upon 13 hypertensive patients (3). Observations have now been made in 88 additional hypertensive patients distributed in several age groups (4–9). The results in these 101 patients have been analyzed to determine whether cerebral oxygen metabolism varies with age, sex, degree of vascular damage elsewhere in the body (10, 11), and with varying arterial and venous blood gas tension.

METHODS

Patients reported in this study were selected according to the following clinical criteria: (*a*) A diastolic blood pressure persistently elevated above 100 mm. Hg; (*b*) damage in one or more vascular beds as evidenced by retinopathy, graded according to Wagener and Keith (11), electrocardiographic and/or fluoroscopic changes of left ventricular hypertrophy and impaired renal function as measured by urea clearance, and intravenous phenolsulphonphthalein excretion; and (*c*) hemoglobin values not less than 12 grams per 100 ml. and hematocrit levels not less than 38 per cent. At the time of these studies, no patient had evidence of mental or neurological disturbances, congestive heart failure, or a blood urea nitrogen persistently above 20 mg. per cent.

Cerebral oxygen consumption ($CMRO_2$) and cerebral blood flow (CBF) were measured by the nitrous oxide method (1), the validity of which we have tested by paired experiments (4).

RESULTS

Table I contains the mean values for the 101 hypertensive patients analyzed for this report. Also tabulated are the standard errors of the cerebral functions measured and arterial and jugular venous blood constituents of the hypertensive group. Data previously reported (1, 12) for normotensive subjects are included for comparison. Cerebral oxygen consumption and CBF in both groups are almost identical. The significant differences ($p < 0.01$) are in mean arterial pressure, cerebral vascular resistance and cerebral respiratory quotient, as noted previously (3).

Variations of $CMRO_2$ with age, Smithwick and Keith-Wagener groups, sex, and blood constituents in these patients are shown in Tables II, III, IV and V. There were no significant deviations in the mean values of any of the sub groups from normotensive values. Cerebral oxygen consumption varies directly with cerebral blood flow ($r = 0.45 \pm 0.08$, $p < 0.01$) and inversely with cerebral vascular resistance ($r = -0.65 \pm 0.07$, $p < 0.01$) (Figure 1). These observations suggest that the $CMRO_2$ does not vary significantly from normal in these selected patients with essential hypertension. More observations are needed in hypertensive patients over the age of fifty who have diastolic as well as systolic hypertension in order to compare their cerebral oxygen uptake with that of aged subjects with no central nervous system disease, who are reported by Fazekas, Alman, and

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TABLE I

Cerebral blood flow, oxygen uptake and blood constituents in 101 patients with essential hypertension: mean values and standard error compared with those of Kety and Schmidt for normotensive males of the third decade

	Hypertension (101)*	Normals (34)*
Cerebral		
Blood flow, *ml. per 100 Gm./min.*	57 ± 1.3	54 ± 2.2
Oxygen uptake, *ml. per 100 Gm./min.*	3.5 ± 0.07	3.3 ± 0.07
Oxygen diff., *ml. per 100 ml.*	6.2 ± 0.11	6.3 ± 0.21
Resp. quotient	0.90 ± 0.01†	0.99 ± 0.02
Vascular resistance (pressure/flow)	2.8 ± 0.08†	1.6 ± 0.07
Arterial		
Oxygen content, *ml. per 100 ml.*	17.2 ± 0.19	18.0
CO_2 content, *ml. per 100 ml.*	50.4 ± 0.56	49.0
pH	7.41 ± 0.005	7.40
pCO_2, *mm. Hg*	42 ± 0.5	40
Mean blood pressure, *mm. Hg*	153 ± 2.1†	86 ± 1.2
Internal jugular		
Oxygen content, *ml. per 100 ml.*	11.0 ± 0.2	11.0
CO_2 content, *ml. per 100 ml.*	56.1 ± 0.55	55.0
pH	7.35 ± 0.005	7.34
pCO_2	51 ± 0.6	51
Oxygen saturation, *per cent*	62 ± 0.6	63
pO_2	35 ± 0.4	36
Hematocrit	43.3 ± 0.3	44.5 ± 6.0‡
Hemoglobin	14.2 ± 0.14	15.0 ± 2.0‡

* Figures in parentheses denote number of observations.
† Denotes significant difference from normal ($p < 0.01$).
‡ Denotes mean values and standard deviations given by Wintrobe.

Bessman to show a decrease (13). Likewise, more observations are needed in hypertensive patients in the second and third decades, to compare with the young subjects having a normal blood pressure (1). Slightly higher values of CBF are evident in hypertensive patients between the ages of 16 and 30 (Table II), but the difference between the mean values for these and normotensive subjects (1) is not significant. Although there is a trend (Table II) for cerebral blood flow to be lower in the older patients in the hypertensive groups, likewise more patients must be studied in order to establish statistically significant differ-

TABLE II

Variation of cerebral oxygen uptake and cerebral blood flow in hypertensive patients of various age decades

Age group	Number of patients	Oxygen uptake		Blood flow	
		ml. per 100 Grams/minute			
		Mean	S.D.*	Mean	S.D.
16–20	2	3.1 ± 0.2		66 ± 6	
21–30	11	3.8 ± 0.7		62 ± 13	
31–40	32	3.7 ± 0.7		59 ± 12	
41–50	47	3.4 ± 0.7		55 ± 13	
51–60	8	3.3 ± 0.6		56 ± 11	
61–70	2	3.2 ± 0.4		50 ± 9	

* S.D.: Standard Deviation.

TABLE III

Variation of cerebral oxygen uptake and cerebral blood flow in hypertensive patients grouped as to severity of the disease

Disease severity grade	Grouped according to the criteria of					
	Smithwick			Keith and Wagener		
	No.	CBF*	$CMRO_2$†	No.	CBF	$CMRO_2$
1	20	58 ± 13‡	3.6 ± 0.8	7	57 ± 11	3.5 ± 0.7
2	30	59 ± 14	3.5 ± 0.6	39	59 ± 14	3.8 ± 0.7
3	16	58 ± 15	3.5 ± 0.8	34	54 ± 14	3.4 ± 0.8
4	35	55 ± 10	3.4 ± 0.7	18	58 ± 10	3.5 ± 0.7

* CBF denotes blood flow in ml. per 100 Grams of brain per minute.
† $CMRO_2$ denotes oxygen uptake in ml. per 100 Grams of brain per minute.
‡ Figures represent mean values with the standard deviation of the individual differences.

TABLE IV

*Variation of cerebral oxygen consumption in men and women with hypertension**

	Number	Oxygen uptake *ml. per 100 Grams/min.*
Men	50	3.4 ± 0.7
Women	51	3.6 ± 0.7

* Figures represent mean values with the standard deviation of the individual differences.

TABLE V

Variation in cerebral oxygen consumption in patients with essential hypertension grouped according to the degree of difference in blood constituents and cerebral respiratory quotient

Mean values and standard deviations of blood constituents in 101 patients		Cerebral oxygen consumption *ml. per 100 Grams/min.*	
		Beyond ±1 S.D.*	Within ±1 S.D.
Arterial			
Oxygen content	17.2 ± 1.9	3.5 ± 0.7 (32)†	3.5 ± 0.7 (66)
Carbon dioxide	42 ± 5	3.5 ± 0.7 (22)	3.5 ± 0.7 (79)
Hydrogen ion	7.41 ± 0.05	3.5 ± 0.7 (17)	3.5 ± 0.7 (74)
Jugular			
Oxygen tension	35 ± 4	3.5 ± 0.7 (13)	4.1 ± 0.7 (21)
Cerebral			
Respiratory quotient	.90 ± 0.12	3.2 ± 0.6 (22)	3.5 ± 0.7 (79)

* S.D.: Standard Deviation.
† Figures in parentheses denote number of observations.

ences. Our mean values (CBF 50 and $CMRO_2$ 3.2) for hypertensive patients aged 60 to 70 are about the same as those reported in normotensive patients of the same age group (2).

The mean values for cerebral arteriovenous oxygen difference, jugular venous blood carbon dioxide tension, oxygen tension and hydrogen ion concentration (Table I) suggest that cerebral ischemia is not a basic disturbance in this group of hypertensive patients having a mean age of 41 ± 9 years, as was reported by Raab after he studied an older group (14). The data of Table I do not support the findings of Waldron and Goldstein (15) that a disturbance in acid-base balance exists in hypertension. We believe the younger mean age of our patients and absence of pulmonary disease may explain the discrepancy. The reduced cerebral respiratory quotient remains unexplained.

Linear distributions are not apparent when the individual observations of mean arterial pressure, arterial oxygen content, arterial carbon dioxide tension, arterial hydrogen ion concentration, jugular venous oxygen tension and cerebral respiratory quotient are plotted graphically against cerebral oxygen consumption. There, also, is not significant variation in the mean values of cerebral oxygen consumption when the patients are grouped according to the degree that the blood oxygen, pH, and CO_2 content vary (Table V).

COMMENT

The measurements of cerebral oxygen metabolism of this and the previous report (3) indicate that the oxygen requirement of the hypertensive's brain is within the range of that of normal subjects and is essentially constant in selected patients of different ages and survival groups who have a normal central nervous system. Despite a mean increase of 75 per cent in cerebral vascular resistance, the blood supply to the brain is automatically adjusted to its oxygen requirements, probably through an intrinsic mechanism which adjusts the cerebrovascular resistance to alterations of perfusion pressure and thus maintains the cerebral blood flow within the normal range (5).

This mechanism of regulating cerebral blood

TABLE VI

Cerebral oxygen uptake in essential hypertension—mean values after various experimental procedures

Procedure	No. of observations	Oxygen uptake	
		Initial	Experimental
Intravenous placebo	6	3.8	3.4
Procaine stellate block	5	3.5	3.0
Differential spinal block	17	3.3	3.2
20° head up tilt	18	3.5	3.8*
Sympathectomy	9	3.5	3.5
Sympathectomy and adrenalectomy	11	3.9	3.9
Depressor drugs			
Dihydroergocornine	12	3.5	3.6
Protoveratrine	15	3.5	3.5
Hydrazinophthalazine	8	3.6	3.2
Hexamethonium†	6	3.6	4.0
Altered arterial gas tension			
5% CO_2	8	3.8	3.6

* Denotes statistically significant increase ($p < 0.01$).
† Data of Crumpton and Murphy, 1952 (18).

flow to meet the oxygen demands does not function perfectly inasmuch as we have observed increased cerebral arteriovenous oxygen differences when cerebral oxygen consumption has not changed or has increased during induced hypotension (5–7). Such observations suggest that the increased cerebral vascular resistance, although reversible, is not capable of complete relaxation under all conditions and that CBF is not precisely adjusted to keep unchanged the cerebral oxygen consumption (Table VI), the arteriovenous oxygen difference, and jugular oxygen tension. The evidence to date suggests that reductions in the latter to 25 mm. Hg or lower may activate chemoreceptors and initiate reflexes to restore cerebral gaseous homeostasis (6).

The few observations in young patients with coarctation of the aorta suggest that cerebral oxygen consumption varies directly with cerebral blood flow. These patients show an increased

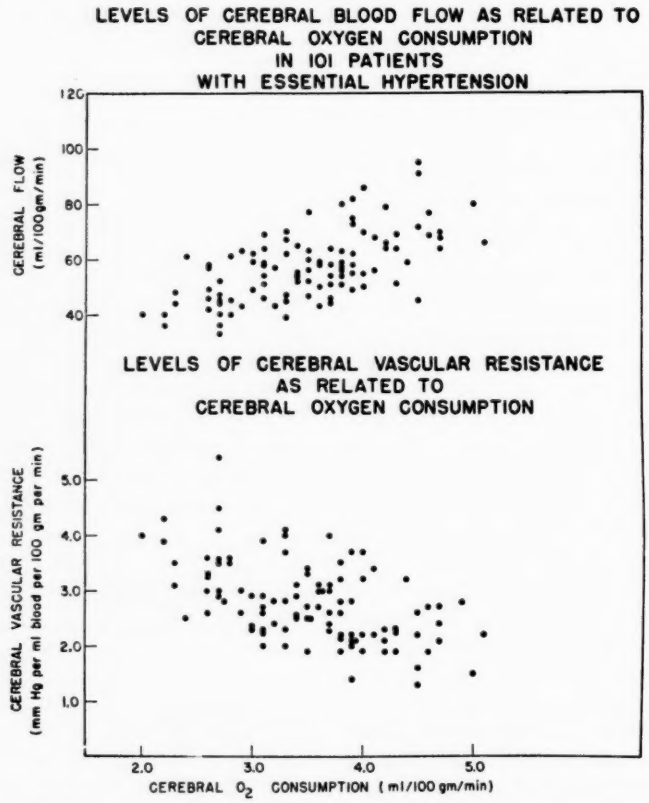

FIG. 1. THE INDIVIDUAL OBSERVATIONS IN 101 HYPERTENSIVE PATIENTS OF CEREBRAL OXYGEN CONSUMPTION HAVE BEEN PLOTTED AGAINST CEREBRAL BLOOD FLOW

Cerebral oxygen consumption varies directly with cerebral blood flow ($r = 0.45$) and inversely with cerebral vascular resistance ($r = -0.65$). The highest cerebral oxygen uptake (5.1) was observed in a 31 year-old female with CBF 66. Three patients were observed with low $CMRO_2$ (2.0 to 2.2) and all had low CBF (36 to 40). These findings confirm the observations of Shenkin *et al.* (2) that levels of cerebral oxygen consumption below those usually found in diabetic coma or pentothal narcosis do occur in conscious subjects without clinical evidence of cerebral impairment.

blood flow and a normal cerebral arteriovenous oxygen difference (16). After the aortic defect was corrected the flow was reduced toward normal and cerebral arteriovenous oxygen difference remained unchanged (17). Further observations in such patients are needed.

The individual observations in CBF and $CMRO_2$ are plotted in the upper half of Figure 1. These data suggest that cerebral blood flow increases as oxygen consumption increases. Reduction of arterial pressure by differential spinal sympathetic block, has disclosed that, despite a reduction in cerebral blood flow, cerebral oxygen uptake remains unchanged as a result of an increased cerebral arteriovenous oxygen difference (6). More recent studies of the effect of 5 per cent carbon dioxide inhalation in hypertensive subjects disclose that the $CMRO_2$ remains constant as the cerebral blood flow increases and the cerebral arteriovenous oxygen difference is reduced (8). Thus the evidence at hand indicates that the cerebral oxygen consumption in patients with essential hypertension is kept constant by a reciprocal relationship between CBF and the cerebral arteriovenous oxygen difference.

Experiments designed to study the effect of reductions in cerebral vascular resistance (Table VI) (4, 5, 7, 8, 9, 18) further suggest that cerebral oxygen consumption remains constant as a result of a reciprocal relationship between cerebral blood flow and cerebral arteriovenous oxygen difference keeping cerebral jugular oxygen tension above 25 mm. Hg. These observations demonstrate how misleading statistically significant coefficients of correlation might be (Figure 1) if causal relationships are assumed and if experiments are not designed to test the suggested hypothesis. The results of our studies add to the growing body of evidence that the cerebral oxygen metabolic rate is about 50 ml. of oxygen per minute, assuming a 1400 gram brain, in both the patients with essential hypertension and in normotensive subjects of various age groups (1, 2, 19).

SUMMARY

Measurements of cerebral blood flow, arteriovenous oxygen difference and cerebral oxygen consumption were made using the nitrous oxide method in 101 selected patients with essential hypertension. Mean values for cerebral oxygen uptake have been calculated for patients grouped as to age, severity of the disease and sex. The data on cerebral oxygen consumption have been plotted graphically against cerebral blood flow, cerebral vascular resistance, cerebral respiratory quotient, mean arterial pressure, arterial oxygen content, carbon dioxide tension, hydrogen ion concentration and jugular venous oxygen tension.

The cerebral oxygen uptake in patients with essential hypertension is the same as that of normotensive subjects. There is likewise no significant variation in oxygen uptake or blood flow in hypertensive patients regardless of age, severity of the disease, sex, or the degree that arterial blood constituents differ.

Cerebral blood flow varies directly with cerebral oxygen consumption and inversely with cerebral vascular resistance, but there is no evidence at present of a causal relationship.

From the data obtained, we believe the following conclusions may be drawn:

The cerebral oxygen consumption in healthy hypertensive patients varies widely but is not significantly different from that of normotensive subjects and does not vary appreciably in hypertensive patients of different sexes, ages or prognosis groups.

The constancy of cerebral oxygen consumption in these studies suggests that the cerebral vessels in hypertension may possess an effective, but somewhat imperfect, intrinsic control in relation to the oxygen metabolic demands of the brain.

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INTRATHECAL FIBRINOLYSIS WITH STREPTOKINASE IN TUBERCULOUS MENINGITIS

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The introduction by Cathie (1) of intrathecal streptokinase to prevent and treat blockage of the cerebrospinal pathways led to hopes that the treatment of this serious complication might be greatly improved; and that prevention and removal of the exudate in this disease might shorten the course of treatment and render chemotherapy more effective. These hopes were sustained by later reports (2, 3). On the other hand, Eadie (4, 5) and Lorber (6), both with equal numbers of treated and control cases, were unable to demonstrate benefit from the use of intrathecal streptokinase. The value of streptokinase in the treatment of tuberculous meningitis is therefore debatable.

The demonstration by Tillett and Sherry (7) that streptokinase produced an active fibrinolytic system in the pleural cavity, and was of great clinical value in the treatment of clotted hemothorax (8) and empyema (9), suggested re-examination of its value in tuberculous meningitis. The success of these authors appeared in part to be due to the fact that they had shown active fibrinolytic enzyme to be present in the pleural fluids of their treated cases, and had used measurements of fibrinolytic activity to assess dosage and control treatment.

A difficulty encountered in the work on tuberculous meningitis has been the proper assessment of dosage to produce free lytic enzyme; in fact no adequate measurements were performed by any of the quoted authors. In view of these uncertainties, clinical trial is not a satisfactory method of approach in deciding the merits or demerits of this treatment, since the immediate clinical assessment of such cases is unreliable, and long term assessment takes at least two years.

A quantitative laboratory investigation of the system employed, therefore, seemed warranted in order to eliminate unfruitful methods of therapy. It has been shown (10, 11) that streptokinase has no fibrinolytic properties but acts on a serum factor named plasminogen to form an enzyme with fibrinolytic properties named plasmin. However, specific antibodies to streptokinase exist in plasma, and may be increased by the injection of this substance, or may follow naturally occurring hemolytic streptococcal infection. The action of plasmin may be partially or completely inhibited by naturally occurring antiplasmins (Figure 1).

Measurements of the components of this system might be expected to supply the information which appears to be essential for the rational control of therapy.

Streptokinase (Lederle) as a consequence of its method of preparation contains a substantial quantity of streptodornase (streptococcal desoxyribonuclease). This enzyme has been shown to exert important therapeutic effects upon purulent exudates containing desoxyribose nucleoprotein (12, 13). Observations in this laboratory made upon meningeal exudate taken from autopsied cases of tuberculous meningitis have shown that this exudate is composed chiefly of fibrin and fibrinous material. Feulgen staining demonstrated comparatively small amounts of nucleoproteins, and *in vitro* experiment confirmed that fibrin lysing systems played a predominant role in the lysis of this material. For these reasons the effects of streptodornase have been ignored, and this communication deals solely with streptokinase action.

Similarly, since the action of the protease inhibititor known to contaminate streptokinase preparations appeared to be quantitatively unimportant in the concentrations of streptokinase used, this factor is not further considered.

Units of measurement

Units of streptokinase and plasminogen were those of Christensen (14). One Christensen unit of plasmin is the quantity of enzyme required to lyse in 30 minutes at 35° C. a fibrin clot derived from 1 ml. of 0.2 per cent bovine fibrinogen. This

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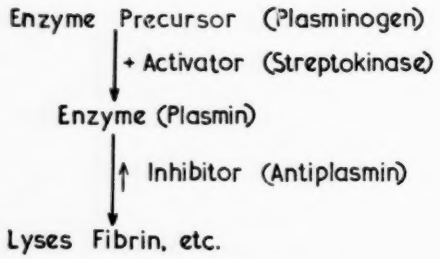

FIG. 1. SCHEME OF SYSTEM

unit will lyse approximately 0.5 mg. of pure human fibrin under the same conditions.

One unit of antiplasmin is the amount of inhibitor that will prevent the fibrinolytic action of 1 unit of enzyme after 10 minutes incubation at 35° C. Experiment showed that 95 per cent of the antiplasmin activity was fixed at the end of this time.

When measuring plasminogen it is assumed, under the favorable experimental conditions employed, that complete conversion to plasmin occurs. The inaccuracies inherent in this assumption and in the method have been stated previously (14).

The system of measurement then becomes:

One unit of plasminogen when activated becomes one unit of plasmin which is inhibited by one unit of antiplasmin.

Assay methods for plasmin involving a substantial degree of dilution will demonstrate enzyme which is not available for fibrinolysis, since the effect of non-specific inhibition is altered by dilution (15). The method of assay for plasmin employed in this investigation requires minimal dilution, and measures only the "free" enzyme available for fibrinolysis with an accuracy ± 10 per cent (15). The unit of enzyme activity is a substantial one, and it is probable that a level of activity equivalent to 1 plasmin unit per ml. C.S.F. is adequate for the requirements of therapy in the cerebrospinal spaces.

METHODS

Plasminogen was estimated by the original Christensen (14) technique, with the modification that, where a more accurate figure was required, a second titration with a narrower range of dilution about the point of 1 unit activity was performed. Plasmin, antiplasmin, and the required dosage of streptokinase were estimated by the methods of Fletcher (15). Streptokinase antibodies were not found to exert much influence in fluids of the cerebrospinal spaces and were not estimated as a routine; when required, a modification of the Christensen (14) technique was used.

RESULTS

Plasminogen content of the cerebrospinal fluid in tuberculous meningitis

Since streptokinase is inactive in the absence of plasminogen, 71 determinations were made on specimens of C.S.F. from 26 cases of tuberculous meningitis. The findings are illustrated in Table I, along with estimations on normal lumbar C.S.F.

It is seen that enzyme precursor is present in much greater amounts than occurs in normal C.S.F. Noteworthy differences in plasminogen content between ventricular and lumbar fluids are present. Cases suffering from spinal block showed in lumbar fluids taken from below the block extremely high plasminogen levels, in some cases as great as those found in the serum. High plasminogen values were found in association with high levels of C.S.F. protein, although the correlation coefficient did not reach the level of statistical significance.

Antiplasmin content

The inhibitor content of 28 specimens of C.S.F. from 18 cases with tuberculous meningitis are recorded together with normal findings in Table II. It is seen that inhibitor is present in every fluid examined, and that significant differences are again apparent between ventricular and lumbar fluids. The inhibitor content of these fluids appears to be low when compared with their plasminogen content. However the interactions of the other components of the system result in these inhibitor contents exerting a significant effect upon the difficulties of inducing fibrinolytic activity.

TABLE I

Plasminogen content of C.S.F.

Site of withdrawal and number of fluids	Average plasminogen content *units/ml.*	Range *units/ml.*
Normal lumbar (10)	6	2– 16
Ventricular (29)	20	2– 64
Lumbar (34)	50	16– 128
Lumbar (8) (spinal block)	500	250–1,000

TABLE II

Inhibitor content of C.S.F.

Site of withdrawal and number of fluids	Average antiplasmin content *units/ml.*	Range *units/ml.*
Normal lumbar (12)	0.5	
Ventricular (12)	1.5	0.5– 5
Lumbar (11)	5	1.5– 8
Lumbar (6) (spinal block)	13	11 –16

Though the antiplasmin content of cerebrospinal fluid tends to rise with increasing protein levels, the correlation does not reach the level of statistical significance.

Transformation of plasminogen to plasmin by streptokinase

The addition of increasing concentrations of streptokinase to biological fluids results in an increase of plasmin activity until with high concentrations a plateau of activity is reached; the lowest concentration of streptokinase inducing the maximal enzyme activity has been designated the "optimal" streptokinase concentration. It has been shown that the "optimal" dose of streptokinase does not result in complete conversion of plasminogen to plasmin (15). These measurements were made on a partly purified plasminogen preparation containing negligible amounts of inhibitor and with an activity corresponding to 5,000 Christensen units per mg.N. Figure 2 shows that when the "optimal" concentration of streptokinase is employed, a high conversion of precursor to enzyme occurs at low precursor concentrations, but that at high precursor concentrations the conversion rate becomes low. The effect of this inefficient transformation of plasminogen to plasmin is to reduce considerably the potential enzyme activity of fluids of high precursor content. This increases the relative importance of the apparently low antiplasmin content of the C.S.F., so that fluids of high plasminogen content have only a small lytic potentiality.

Maximal plasmin activity

A number of factors unfavorable to the appearance of "free" fibrinolytic enzyme have been discussed. It was thought that these factors, the presence of streptokinase antibodies, the inefficiency of streptokinase activation, and the content of anti-

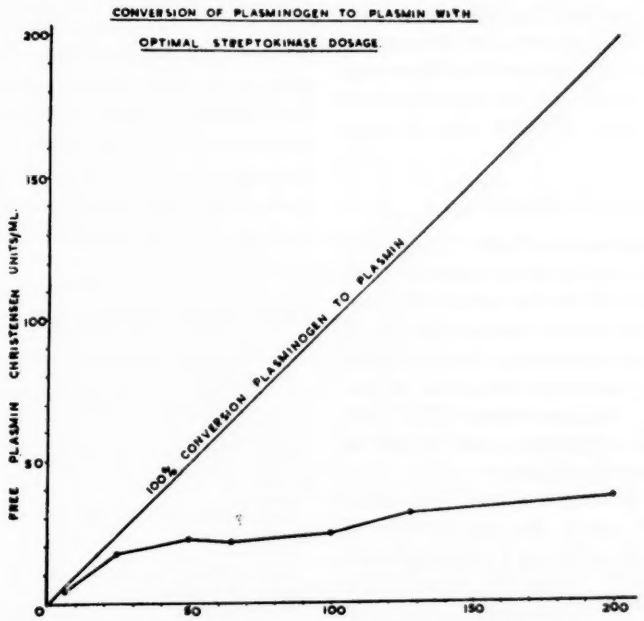

FIG. 2. AVAILABLE PLASMINOGEN CHRISTENSEN UNITS/ML.

TABLE III

Plasmin levels with optimal streptokinase

Site of withdrawal and number of fluids	Range of enzyme potency plasmin *units/ml.*
Normal lumbar (10)	0.25–1.5
Ventricular (10)	1 –3
Lumbar (19)	1.5 –5
Lumbar (spinal block) (7)	1 –2.5

plasmin might result in a complete suppression of fibrinolytic activity even with optimal amounts of streptokinase. Titrations of 56 cerebrospinal fluids from 23 patients were made with varying amounts of streptokinase; in each case free fibrinolytic enzyme was shown to be present provided that a suitable amount of streptokinase was added to the fluid. It was found that where insufficient streptokinase was used, the unfavorable factors resulted in a complete suppression of fibrinolytic activity. This implies a minimum streptokinase concentration below which physiologically active enzyme is not released, and below which clinical effects attributable to fibrinolysis cannot be expected. Table III shows the maximum enzyme ranges recorded in 36 cerebrospinal fluids from 18 patients where the "optimal" amount of streptokinase was employed. It is seen that despite the wide variations in plasminogen and inhibitor content demonstrated previously, the potential lytic potency of fluids from different sites is very similar.

The clinical requirement of streptokinase

The C.S.F. enzyme levels in Table III were obtained using streptokinase concentrations of 25 to 100 units per ml. C.S.F. for the ventricular and lumbar fluids, and 100 to 500 units per ml. C.S.F. for the lumbar fluids taken below spinal blocks. These streptokinase concentrations would be unsafe to use clinically, since assuming a C.S.F. volume of 120 ml. the intrathecal dose would be 2,500 to 15,000 units streptokinase.

It was decided to investigate a lower level of activity, and Table IV shows the concentration of streptokinase required to induce 1 unit of plasmin activity per ml. C.S.F. Table IV shows that a lower concentration of streptokinase is required to activate ventricular fluid than lumbar fluid; this difference is in line with the other differences in composition noted in Tables I to III. Ventricular fluids required 1.5 to 30 units streptokinase per ml. C.S.F. to produce an enzyme potency of 1 unit per ml. C.S.F., lumbar fluids required 3 to 60 units streptokinase per ml. C.S.F., while lumbar fluids from below spinal block required 30 to 120 units streptokinase per ml. C.S.F. to produce the same lytic potency. For 45 out of 58 fluids the ineffective streptokinase concentration was one step below that required for 1 unit activity, *i.e.*, if a fluid required 10 units streptokinase per ml. C.S.F. for 1 unit of enzyme activity, 6 units streptokinase per ml. C.S.F. produced no free enzyme. For 17 fluids the ineffective dose was two steps below the dose required for 1 unit of enzyme activity (see Table IV).

These differences in composition between ventricular and lumbar fluids render it difficult to calculate the clinical dose. Since cisternal fluid more nearly resembles ventricular than lumbar fluid, the dose for each patient was calculated on the assumption that the ventricular fluid assay represented a true sample of 80 ml. C.S.F. and the lumbar fluid represented a sample of 40 ml. The use of this simplification suggested that where the cerebrospinal circulation was free, 90 per cent of patients would require an intrathecal dose varying between 300 and 3,000 units of streptokinase; in practice the dose was calculated individually for each patient. Though fluids with a high protein content required large doses of streptokinase for their activation, and fluids of low protein content small doses, the correlation coefficient did not reach the level of statistical significance.

TABLE IV

Number of fluids showing at least 1 unit of plasmin activity with various streptokinase concentrations

Streptokinase *units/ml.*	0	1.5	3.0	6.0	10	15	30	60	120
Number of ventricular fluids (24)	0	3	11	15	22	23	24		
Number of cisternal fluids (10)	0	2	5	7	10				
Number of lumbar fluids (18)	0	0	3	5	10	15	15	18	
Number of lumbar fluids below spinal block (8)	0	0	0	0	0	0	1	3	8

Correlation between in vivo and in vitro enzyme activity

Where a number of unknown factors exist, calculations made from *in vitro* testing must be confirmed by *in vivo* experiment. It was realized in making this comparison that inaccuracies connected with sampling and estimation of C.S.F. volumes had occurred, but it was believed that the results were sufficiently informative to be valuable.

Table V shows the calculated enzyme activity, the calculated streptokinase concentration, and the enzyme activity obtained by sampling.

The calculated *in vivo* enzyme level was ascertained by *in vitro* titrations on samples of ventricular and lumbar fluids from the patient, activated with varying quantities of streptokinase. Suitable concentrations of streptokinase to give equal activities in lumbar and ventricular samples having been found, the dose for injection was calculated on the assumption that ventricular fluid represented a volume of 80 ml. C.S.F. and lumbar fluid represented a sample of 40 ml. C.S.F. The calculated *in vivo* streptokinase concentration in units per ml. was then obtained by dividing the total dose of streptokinase by 120.

When the ventricular or cisternal route was used for injecting the streptokinase, ventricular and cisternal samples were withdrawn at 10 and 30 minutes after injection from both sites. The highest level of enzyme activity obtained from these samples was regarded as the *in vivo* enzyme level. A few samples were obtained two hours after injection, but these samples invariably showed much less activity than earlier samples. In cases where streptokinase was injected below a spinal block, the volume of the fluid below the block was determined by the phenolsulphonephthalein dilution method, and the dose of streptokinase was found using this volume as a basis for calculation.

Though the error of sampling by these procedures would be very large, earlier work (15) had shown that the quantity of enzyme activity produced in C.S.F. was not proportional to the concentration of streptokinase, but approximately proportional to the log concentration of streptokinase, and this relationship would do much to minimize errors due to incomplete distribution.

It might be expected that adsorption of fibrinolytic enzymes onto fibrin would occur *in vivo* and thus give rise to spuriously low levels of enzyme on sampling. The importance of this factor was investigated by means of *in vitro* tests with models.

It can be calculated that in the spinal meninges, the maximum ratio of meningeal surface to C.S.F. volume is approximately 4 sq. cm. of surface to 1 ml. of C.S.F. The general ratio of meningeal surface to C.S.F. volume is much less than this, and in addition only a part of this surface would be covered with fibrin. Glass tubing of 4 mm. bore provides a surface to volume ratio of 1 sq. cm. per 1 ml. Thirty cm. lengths of 4 mm. glass tubing were coated with fibrin by introducing 0.4 ml. of fibrinogen thrombin mixture (containing 12 mg. bovine fibrinogen) along the bore of the tube with a fine pipette and rotating the tubes rapidly in the chuck of an electric motor until clotting had occurred. Assays conducted on samples of streptokinase activated C.S.F. respectively incubated in fibrin coated and similar glass tubes revealed negligible differences over the range 3.0 to 0.3 units per ml. plasmin. It was concluded that over this range of enzyme activity adsorption onto fibrin *in vivo* would not increase the sampling error.

Table V shows that a great disparity exists between the enzyme level calculated on the basis of *in vitro* testing and the enzyme level actually found *in vivo*. For instance, the first line of the table shows that in a fluid taken below a spinal block

TABLE V

Enzyme assays of C.S.F. after intrathecal injection of streptokinase. Comparison with levels suggested by in vitro testing

Calculated *in vivo* streptokinase concentration *units/ml.*	Calculated *in vivo* enzyme level (from *in vitro* testing) *units/ml.*	Actual *in vivo* enzyme level determined by sampling *units/ml.*	
500	2.6	<0.1	spinal blocks
200	3.0	<0.1	
200	1.7	<0.1	
50	0.5	<0.1	
50	1.4	<0.1	
50	2.8	<0.1	
50	3.2	<0.1	
34	1.9	<0.1	
30	2.2	0.5	
25	1.3	0.8	
20	1.8	1.0	
10	1.5	0.5	
8	2.0	1.0	
5	1.0	0.8	

the calculated streptokinase level was 500 units per ml. which on *in vitro* testing should have provided an *in vivo* level of 2.6 enzyme units per ml. The actual level as determined by *in vivo* sampling was < 0.1 units per ml. In cases where large doses of streptokinase were injected below spinal blocks no free fibrinolytic enzyme was produced, and in fact in cases where large doses of streptokinase were shown to be necessary by *in vitro* testing the enzyme level was most disappointing. In cases where *in vitro* testing had indicated that a small dose of streptokinase could be used with success, the disparity between the calculated and the actual enzyme level found on sampling was not great; for instance the bottom line of Table V shows that the calculated enzyme level with 5 units of streptokinase per ml. C.S.F. was 1 unit per ml., and the actual level found on sampling was 0.8 unit per ml. It is apparent that the use of high concentrations of streptokinase in the spinal theca frequently results in most disappointing enzyme levels, while the use of low concentrations of streptokinase is usually much more successful.

The reasons for this discrepancy are interesting and important. The evidence points strongly to the fact that streptokinase in high concentrations exerts an irritative effect upon the spinal theca resulting in the in-flow of serous fluid with a resulting high concentration of inhibitor. Assays in four cases, where free fibrinolytic enzyme had not been demonstrated after high streptokinase dosage, showed that the antiplasmin value had increased over 40 per cent in each case. Such an increase in inhibitor would account for the fact that fibrinolytic enzyme could not be demonstrated *in vivo*. Though it was realized that fresh plasminogen would also enter the C.S.F. with the inhibitor, inspection of the data in Tables I and III shows that this material would exert comparatively little effect. The practical value of this observation is clear; it indicates that the intrathecal injection of streptokinase may exert, in the absence of demonstrable clinical upset, an irritative effect upon the spinal theca, which is sufficient to nullify its potential therapeutic action.

The effect of a single streptokinase injection upon the C.S.F. plasminogen level

Since the effect of streptokinase is to convert enzyme precursor to enzyme, there may be a fall in precursor level following an injection of streptokinase. Table VI shows the extent of the fall and the rate of recovery to the former plasminogen level; these observations refer to patients with a free cerebrospinal circulation. It is seen that though an early fall in level is apparent, this fall is temporary, and the level has largely returned to its former state in 24 hours. These findings suggest that if a period of 24 hours is allowed to elapse between the first and the next streptokinase injection, the effect of the last injection will not be hindered by lack of precursor. In patients with various types of cerebrospinal blockage the situation is far from simple, and the results in such cases have not been tabulated owing to their inconstant pattern and the multiplicity of factors at work.

TABLE VI

Plasminogen level following streptokinase injection

Initial plasminogen level	Level 6 hours after streptokinase	Level 24 hours after activation
256	64	128
128	64	256
128	4	32
64	4	32
32	2	16

DISCUSSION

Streptokinase may be employed under three distinct circumstances in the therapy of tuberculous meningitis, and these circumstances profoundly influence the nature of its employment and the chances of success with its use. These uses are (*a*) prophylactically before obstruction of the cerebrospinal pathways has occurred, (*b*) for the therapy of established or pending subtentorial and basal cistern block and (*c*) for the therapy of spinal block.

Streptokinase has been employed as a prophylactic measure in therapy by most workers. The findings reported here demonstrate that the routine use of a dose of 100 Christensen units (600 Cathie units) streptokinase will fail to produce free enzyme in a substantial proportion of cases. The optimal dose for intrathecal treatment appears to lie in the range 300 to 3,000 units, with the majority of patients requiring doses at the lower end of the range. Unfortunately the optimal dose can only be gauged by laboratory assay. It is how-

ever certainly possible, and probably desirable, to use streptokinase in this manner provided its use is controlled by laboratory assay.

The investigations of Lorber (16) have revealed the high incidence and dire consequences of subtentorial block, and it has also been our personal experience that this complication is responsible for much mortality and morbidity.

Despite the recognition of this complication clinically, and the possibility of controlling the resultant hydrocephalus by ventricular drainage, the end results of treatment are poor. It would appear that streptokinase may prove effective in lysing the fibrin producing the block, since the enzyme can be placed in close proximity to the lesion by cisternal puncture, and assays on cisternal fluid have shown that lytic activity can be induced by small doses of streptokinase. The wisdom of leaving treatment with lytic factors to this stage is doubtful.

The easily diagnosed complication of spinal block exerts a considerable fascination for those wishing to assess the effect of fibrinolytic therapy owing to the ease with which the progress of treatment can be ascertained. Assay of lumbar fluids taken from below spinal blocks has demonstrated that the problem presents peculiar difficulties. The antiplasmin content of the lumbar fluids ranged from 11 to 16 units; the dose of streptokinase which fails to produce free enzyme may be as high as 34 units streptokinase per ml. C.S.F.; the concentration required to produce 1 unit of plasmin per ml. may be double this figure, while the range of dose for optimal activation is higher still. *In vivo* assay has previously shown that unsatisfactory results occur when dosage of this order is suggested by *in vitro* testing. The clinical experience may be briefly summarized by the statement that 25 attempts to lyse nine spinal blocks have been made and only one temporary success resulted. It was concluded that streptokinase alone on the basis of *in vitro* testing and clinical trial is an unsatisfactory agent for the treatment of spinal block.

SUMMARY

Examination of the components of the plasminogen plasmin system present in the cerebrospinal fluids of patients suffering from tuberculous meningitis suggests that the injection of streptokinase is capable of inducing useful concentrations of fibrinolytic enzyme. The concept of a lower limit of effective dosage below which, owing to the presence of inhibitors, no free fibrinolytic enzyme is produced, has been advanced. It has also been shown that an upper limit of intrathecal dosage is also present, since not only will high concentrations of streptokinase produce systemic upsets, but the local irritation of the spinal theca will result in the failure of such therapy. Laboratory examination of the cerebrospinal fluid is essential to the estimation of the correct intrathecal dose.

It has been shown that the customary dose of streptokinase used for intrathecal treatment (100 Christensen Units) will usually be insufficient to produce free fibrinolytic enzyme. The variation between individual patients is considerable, but the optimal dose for intrathecal treatment usually lies between 300 to 3,000 units of streptokinase; a standard dose cannot be stated. It is believed that these experiments indicate the reason for the variable clinical results obtained by various workers.

These investigations indicate that streptokinase may be used with reasonable chances of success, either prophylactically, or for the treatment of subtentorial or basal cistern block; the chances of successful treatment of spinal block by this means are small.

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THE ANTIDIURETIC ACTIVITY OF PLASMA OF PATIENTS WITH HEPATIC CIRRHOSIS, CONGESTIVE HEART FAILURE, HYPERTENSION AND OTHER CLINICAL DISORDERS [1]

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The retention of water which occurs in hepatic cirrhosis and in congestive heart failure has been attributed by some investigators to an increase in the concentration of circulating antidiuretic hormone of the neurohypophysis. In support of this hypothesis are the reports of a diminished diuretic response to the ingestion of water (1, 2) and of an increased antidiuretic activity of the blood or urine of patients with ascites and edema. Critical examination of such reports, however, reveals that the assay methods employed usually were neither sensitive nor precise (3) or that the number of subjects studied was too small for statistical evaluation (Table I).

The availability of a relatively simple, sensitive and precise bioassay in the rat for the measurement of antidiuretic activity (4) prompted the examination of the activity of the blood of patients with the aforementioned as well as with other clinical disorders.

METHOD AND RESULTS

Only men were selected for this study in order to obviate any possible influence of the menstrual cycle. The subjects selected to comprise the "healthy" group showed no apparent physiological or psychological disturbances. All patients with cirrhosis of the liver had ascites and edema. The patients with congestive heart failure had from ++ to ++++ edema. The blood pressures of the patients with hypertension were all beyond a systolic of 180 mm. mercury and a diastolic of 100 mm. mercury.

After a preliminary period of ten hours during which the subjects were not permitted to ingest food or water, venous blood samples were drawn. The plasma was separated rapidly by centrifugation and assayed within one hour by the procedure described by Stein, Jinks, and Mirsky (4). Since previous studies revealed that the slope of the dose-response curve for plasma was essentially the same as that for pitressin and that this slope was fairly constant, only one dose of pitressin was used as the standard in each assay and the concentration of antidiuretic stubstance (ADS) in the plasma computed and expressed in terms of pitressin.

In brief, the assay procedure was as follows: After a preliminary fast of approximately 16 hours during which time the rats were permitted free access to water, 5 ml. of warm 0.2 per cent NaCl per 100 grams body weight were administered by stomach tube. One hour later, the gavage was repeated, and twenty minutes after the second gavage, 1 ml. of plasma per 100 grams body weight or a similar volume of a solution containing 0.6 mU of pitressin in isotonic saline was injected intraperitoneally. The animals were handled and prodded every twenty minutes after the second gavage, and the volume of urine excreted at the 20 and 60 minute intervals was recorded. The response was expressed as the reciprocal of the ratio of the volume of urine excreted by 60 minutes to that excreted by 20 minutes after the second gavage. The mean response of five rats in individual metabolic cages was utilized to calculate the ADS potency of the plasma.

The antidiuretic activity of the plasma of 19 healthy men and of 108 men suffering from a variety of clinical disorders is depicted in Figure 1 and summarized in Table II. In the healthy group of men, the mean ± S.E. antidiuretic activity of the plasma, expressed as pitressin equivalents, was 24.1 ± 3.9 mU per 100 ml. The individual values distribute themselves along a normal curve. The values for the patients suffering from various clinical disorders, however, vary markedly and are not distributed normally. Whereas the mean ADS value for each clinical disorder is greater than that of the healthy group, the median is quite similar to that of the healthy group (Table II). Accordingly, the majority of patients within each clinical group have values which are similar to those of the healthy individuals. Likewise, the plasma ADS values of approximately two-thirds of the subjects comprising all the clinical groups are within the range of those of healthy men.

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DISCUSSION

The procedure utilized in this study for the measurement of the antidiuretic activity of the plasma permits the detection of from 0.1 to 1.2 mU of pitressin per ml. Consequently, many of the values obtained in normal men are just within the lower limits of the method. Since there are no other data in the literature in which the antidiuretic activity of the plasma is expressed in terms of pitressin equivalents, it is impossible to compare the range of values reported herein with those of other observers. The use of pitressin as a standard in the bioassay procedure and the quantitative expression of the antidiuretic activity of the plasma in terms of pitressin equivalents does not imply that the antidiuretic activity of the plasma is due to endogenous vasopressin.

The data reported herein do not support the hypothesis that the retention of water by patients with cirrhosis of the liver or by patients with congestive heart failure is related to an increase in the antidiuretic activity of the blood. Only 28 per cent

TABLE I

Summary of reports on the antidiuretic activity of blood and urine in various clinical disorders

Disease entity	Body fluid	Results*	Reference
Cirrhosis	Urine	+ ADS in patients with ascites 0 ADS in patients without ascites 8 patients and 4 normals studied	Ralli, Robson, Clarke, and Hoagland (5)
Cirrhosis	Urine	+ ADS in 4 patients + ADS in rats with or without liver damage	Hall, Frame, and Drill (6)
Cirrhosis	Urine	+ ADS in 50% of 22 patients with ascites + ADS in 35% of 22 patients without ascites	VanDyke, Ames, and Plough (3)
Cirrhosis	Blood (serum)	+ ADS in 75% of 4 patients	Lloyd and Lobotsky (7)
Cirrhosis	Urine	+ ADS in 93% of 15 patients with ascites 0 ADS in non-ascitic liver disease	Sims (8)
Hepatitis	Urine	+ ADS in 8 patients during early icteric stage + ADS decreased markedly during convalescence	Labby (9)
Congestive heart failure	Urine	+ ADS in 80% of 15 patients	Bercu, Rokaw, and Massie (10)
Congestive heart failure, Cirrhosis, Addison's disease	Urine	+ ADS in 12 patients with congestive heart failure + ADS in 2 patients with cirrhosis + ADS in 2 patients with controlled Addison's disease	Dochios and Dreifus (11)
Congestive heart failure, Cirrhosis, Addison's disease	Blood (serum)	No significant difference between ADS of controls and patients in failure or with liver disease. No correlation between ADS and edema or with liver damage. 0 ADS in 3 patients with controlled Addison's disease	Perry and Fyles (12)
Hypertension	Urine	+ ADS in 73% of 15 patients	Ellis and Grollman (13)
Hypertension	Blood (serum)	+ ADS in 46% of 56 patients + ADS also in patients without hypertension, *i.e.*, malignant tumors, pregnant women, and one patient with recurring duodenal ulcer	Griffith, Corbit, Rutherford, and Lindauer (14)
Bright's disease, Cushing's disease, Premenstrual tension, Nephrosis	Urine	Correlation between clinical edema and + ADS. However, found some edematous patients with 0 ADS and + ADS in patients without edema.	Robinson and Farr (15)
Cirrhosis, Congestive heart failure, Hypertension, Miscellaneous	Blood (plasma)	+ ADS in 28% of 25 patients with cirrhosis with ascites + ADS in 18% of 28 patients with congestive heart failure + ADS in 36% of 25 patients with hypertension + ADS in 43% of 30 patients with miscellaneous entities	This report

* 0 ADS—same as controls; + ADS—greater than controls.

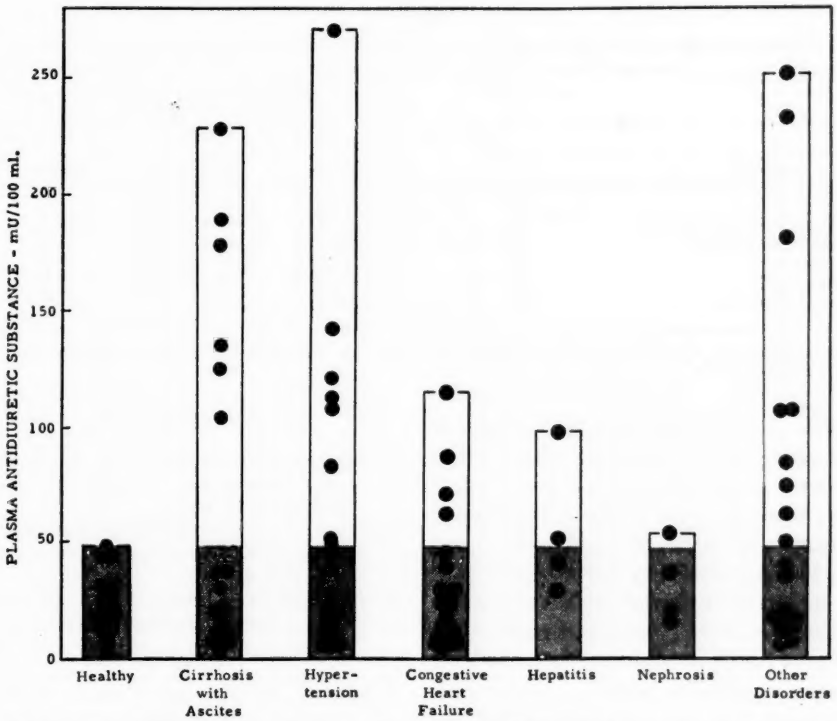

FIG. 1. THE ANTIDIURETIC ACTIVITY OF PLASMA FROM HEALTHY MEN AND FROM PATIENTS WITH VARIOUS CLINICAL DISORDERS

of 25 patients with ascites showed an antidiuretic activity of the plasma which was beyond the range of values found in the healthy men. Likewise, the plasma ADS was increased in only 18 per cent of 28 patients with the edema of congestive heart failure. Four patients with nephrosis showed no significant deviation from the control group.

In accord with the conclusion that there is no relationship between the retention of water and the antidiuretic activity of the blood are the observations of Van Dyke, Ames, and Plough (3) that the antidiuretic activity of the urine was increased in 50 per cent of 22 patients with the ascites of hepatic cirrhosis and in 35 per cent of similar patients without ascites. Likewise, Perry and Fyles (12) found no correlation between the antidiuretic activity of the blood serum of patients with liver disease and the presence of edema.

From their observation that the antidiuretic activity of the urine was increased in 73 per cent of 15 patients with hypertension, Ellis and Grollman (13) postulated some relationship between this syndrome and the presence of a high antidiuretic activity. Griffith, Corbit, Rutherford, and Lindauer (14) noted an increased antidiuretic activity of the blood serum in 46 per cent of 56 patients with hypertension. In the present study, an elevated antidiuretic activity of the plasma was found in 36 per cent of 25 patients with hypertension. Consequently, it appears that there is no significant relationship between the presence of hypertension and the antidiuretic activity of the blood.

The plasma ADS was increased in 43 per cent of patients with conditions other than cirrhosis of the liver, congestive heart failure, and hypertension. When all the individual disorders are combined, a similar increase is found in 31.5 per cent of the 108 patients. A chi square analysis reveals no significant difference between the incidence of patients with an increased plasma ADS in the various clinical states or between the incidence in any particular syndrome and in the combination

TABLE II

The antidiuretic activity of the plasma of healthy men and patients with various clinical disorders

Diagnosis	No.	Antidiuretic activity—pitressin equivalents *mU/100 ml.* Range	Mean ± S.E.	Median	Per cent beyond 'Healthy'
Healthy	19	3.6– 48.2	24.1 ± 3.9	21.1	—
Hepatic cirrhosis with ascites	25	3.5–228.0	55.5 ± 65.4	21.2	28.0
Congestive heart failure	28	3.1–114.8	29.8 ± 32.0	13.9	17.9
Hypertension	25	6.3–270.0	57.2 ± 63.4	38.2	36.0
Miscellaneous*	30	7.4–252.5	58.3 ± 58.3	39.5	43.3
All clinical disorders	108	3.1–270.0	50.4 ± 56.0	27.0	31.5

* Acute nephritis (2), Cor pulmonale (4), Gastric carcinoma (2), Hepatitis (4), Lupus erythematosis (1), Psychoneuroses (6), Psychoses (7).

of syndromes. Accordingly, the common denominator responsible for the augmented plasma ADS cannot be some specific physiological distortion.

The only apparent factor which is common to all the patients is the presence of an illness. Consequently, the degree of non-specific stress produced by the patient's physiological derangement or by the character of the psychological response to the presence of his illness may determine the level of the plasma antidiuretic activity. Either possibility is supported by the demonstration that exposure to a noxious stimulus results in an increase in the antiduretic activity of the blood plasma of both man and lower species (16).

SUMMARY

The antidiuretic activity of the blood plasma was measured in healthy men and in men suffering with the ascites and edema of cirrhosis of the liver, with the edema of congestive heart failure, with hypertension, and with a variety of other clinical disorders.

The incidence of an augmented antidiuretic activity of the plasma was 28 per cent in patients with the ascites of hepatic cirrhosis, 18 per cent in patients with the edema of congestive failure, 36 per cent in patients with hypertension, 43 per cent in patients with a variety of other clinical disorders, and 32 per cent in all the subjects with clinical disorders. Consequently, there was no relation between the retention of water and the augmentation of the antidiuretic activity of the blood.

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THE RENAL RESPONSE TO ACUTE RESPIRATORY ACIDOSIS[1]

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It is well known that patients suffering from acute and chronic ventilatory insufficiency or in whom there exists an impaired diffusion of carbon dioxide across the alveolo-capillary membrane may develop an acid-base disorder (respiratory acidosis) characterized by decrease in arterial pH and elevation of the total CO_2 content of the arterial blood (1–5). This syndrome of carbon dioxide retention has been noted clinically in patients with pulmonary emphysema, tracheal or laryngeal obstruction, during the coma of severe barbiturate and morphine poisoning, and in both acute paralytic and convalescent phases of poliomyelitis, where the common factor is failure of CO_2 elimination by the lungs.

Previous studies (6, 7) indicate that carbon dioxide is buffered by body buffers, principally hemoglobin of whole blood, and that the increase in plasma bicarbonate concentration characterizing respiratory acidosis occurs more or less in proportion to the degree of CO_2 retention. This increase is compensatory, minimizing the fall in blood pH which would otherwise ensue.

The inhalation of carbon dioxide mixtures has been shown to produce a diuresis with decrease in urine osmolarity, and increase in excretion of ammonia and titratable acid (8, 9). Since the plasma bicarbonate concentration is elevated in consequence of the buffering of retained carbon dioxide, one would anticipate from *a priori* considerations that a part of the renal compensation in acute respiratory acidosis would consist in an enhancement of the tubular reabsorption of bicarbonate bound base so as to maintain plasma bicarbonate concentration at supranormal levels.

The studies reported below indicate that acute respiratory acidosis induced by respiring carbon dioxide-oxygen mixtures does in fact result in enhancement of the renal reabsorption of bicarbonate bound base, and that the elevation of carbon dioxide tension of the body fluids rather than lowering of pH is primarily responsible for this increase. Additional data to be presented suggest that bicarbonate bound base is reabsorbed by a process of ion exchange.

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METHODS

A total of 39 experiments was performed on 12 mongrel female dogs. Intravenous sodium pentobarbital was used to induce and maintain anesthesia; dosage was variable, but did not exceed 30 mg. per Kg. In all experiments the dog was intubated with an endotracheal airway equipped with an inflatable rubber cuff. During control periods the dog respired ambient room air. Mixtures of CO_2 and O_2 were administered as desired by connecting the endotracheal tube to a non-rebreathing Digby-Leigh valve, the inspiratory side of which was connected to a Douglas bag containing the mixtures to be breathed. The dogs breathed spontaneously throughout; artificial respiration was not employed. This arrangement insured the maintenance of an adequate airway and eliminated the necessity of a tracheotomy, thereby permitting repeated studies to be performed on each animal.

Solutions were infused intravenously at desired rates using a calibrated pump. Urine samples were collected using a Foley catheter connected to a length of rubber tubing which dipped under mineral oil in the collecting graduate. Exposure to air with resultant loss of CO_2 was thereby minimized. Each collection period was terminated by manual compression of the abdomen, facilitating bladder emptying. Arterial blood samples were obtained at the midpoint of each period from an indwelling needle in the femoral artery. One portion, collected with heparin as the anticoagulant, was used for the determination of creatinine, sodium and potassium. A second portion was collected in an oiled syringe containing two drops of saturated potassium oxalate. Two cc. were delivered into a Van Slyke pipette for determination of pH; the remainder was delivered under mineral oil into a tube, which was then sealed with paraffin, centrifuged and analyzed for CO_2 content within two hours. Creatinine was determined in urine and protein-free filtrates of plasma precipitated with tungstic acid, by the method of Phillips (10). Total CO_2 in plasma and urine was determined by the manometric extraction technique of Van Slyke and Neill (11). The pH of whole blood and urine was determined using a water-jacketed Cambridge condenser-type glass electrode, at a uniform temperature of 37°C. Concentrations of bicarbonate and dissolved CO_2 were calculated from the Henderson-Hasselbach equation, employing a pK′ for

TABLE I

Experiments illustrating the effect of acute respiratory acidosis on the renal reabsorption of bicarbonate bound base

Concurrent time	Gas respired	Glom. filt. rate	Urine flow	Plasma			Urine			$BHCO_3$			
				pH	$BHCO_3$	pCO_2	pH	$BHCO_3$	pCO_2	Filt.	Excr.	Reabsorbed	
Minutes		*ml./min.*	*ml./min.*		*mEq./L.*	*mm. Hg*		*mEq./L.*	*mm. Hg*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./100 ml. filtrate*
Experiment No. 5. Dog B—20.9 Kg.													
−82		Start infusion: Creatinine 2.66 Gm./L.; $NaHCO_3$ 16.6 Gm./L. @ 5.5 ml./min.											
−78		Prime: Creatinine 1.3 Gm.; $NaHCO_3$ 5.3 Gm.											
0–20	Room	99.9	2.23	7.58	32.9	36.2	8.10	266	87.4	3.29	0.59	2.70	2.70
20–40	Air	90.0	2.06	7.57	34.1	38.6	8.14	250	74.4	3.08	0.52	2.56	2.86
55–75	12% CO_2–	104	2.50	7.33	38.9	76.1	7.94	221	104	4.05	0.55	3.50	3.36
75–95	88% O_2	104	3.00	7.33	40.2	78.6	7.92	210	104	4.19	0.63	3.56	3.42
110–130	Room	104	4.29	7.54	36.7	44.2	8.04	247	90.6	3.80	1.06	2.74	2.65
130–150	Air	101	4.10	7.49	37.0	50.2	8.05	247	90.6	3.74	1.01	2.73	2.70
Experiment No. 8. Dog A—15.7 Kg.													
−80		Start infusion: Creatinine 2.3 Gm./L.; $NaHCO_3$ 18.0 Gm./L. @ 5.0 ml./min.											
−73		Prime: Creatinine 1.5 Gm.; $NaHCO_3$ 5.0 Gm.											
0–20	Room	63.5	3.30	7.48	41.4	57.1	7.96	256	113	2.76	0.84	1.92	3.02
20–40	Air	62.4	3.30	7.45	41.8	61.8	7.99	261	110	2.74	0.86	1.88	3.02
75–95	12% CO_2–	65.0	2.75	7.29	45.4	87.4	7.89	254	133	3.10	0.70	2.40	3.71
95–115	88% O_2	65.2	3.23	7.30	45.2	94.9	7.85	255	146	3.10	0.82	2.28	3.49
130–150	Room	75.1	4.52	7.48	42.1	58.5	7.89	252	133	3.33	1.14	2.19	2.92
150–170	Air	72.7	4.25	7.44	42.2	64.2	7.96	252	113	3.22	1.08	2.14	2.94

carbonic acid of 6.1, and 0.0591 α equal to 0.0301 for plasma and 0.0309 for urine. It was not considered necessary to adjust these factors for the ionic strength of plasma or urine. Sodium and potassium concentrations in plasma and urine were determined using an internal standard flame photometer.

The creatinine clearance was taken as equal to the glomerular filtration rate. The amount of each ion filtered in unit time was calculated as the product of its plasma concentration and the glomerular filtration rate, corrected for Donnan distribution across the glomerular membrane. A factor of 0.95 was taken for Na^+ and K^+, and 1.05 for Cl^- and HCO_3^-. The rate of excretion was calculated as the product of urine concentration and urine flow. The rate of reabsorption of each ion was calculated as the difference between the rates of filtration and excretion. Since in the dog and in man the reabsorption of bicarbonate per unit time has been shown to be a rectilinear function of glomerular filtration rate (12), the rate of reabsorption was also expressed in terms of the amount reabsorbed per 100 ml. of filtrate. This was done by dividing the absolute rate of reabsorption (mEq. per min.) by the GFR (ml. per min.) and multiplying by 100.

RESULTS

The effect of acute respiratory acidosis induced by breathing 12 per cent CO_2–88 per cent O_2 on the renal reabsorption of bicarbonate bound base was determined in eight experiments. Data from two representative experiments of this group are presented in Table I. In these experiments, the plasma concentration of bicarbonate was elevated to a level adequate to insure frank excretion and was maintained at approximately 37 to 44 mEq. per liter by infusing sodium bicarbonate at a constant rate. During the first two periods the dog respired room air; this was changed to 12 per cent CO_2–88 per cent O_2 during the middle two periods, and the dog again respired room air during the final two periods. Plasma pH, initially elevated by the bicarbonate infusion, dropped during the inhalation of CO_2, returning to control levels when air breathing was resumed. Plasma pCO_2 rose from essentially normal values on room air to approximately 90 mm. Hg [3] during CO_2 breathing, to fall toward control values when air was restored.

The final two columns of this table demonstrate the effect of acute respiratory acidosis on the re-

[3] Variability in pCO_2 in animals breathing 12 per cent CO_2 is the result of a number of factors including: leakage of room air into the system, differences in composition of tank gas, and variable respiratory depression from anesthesia.

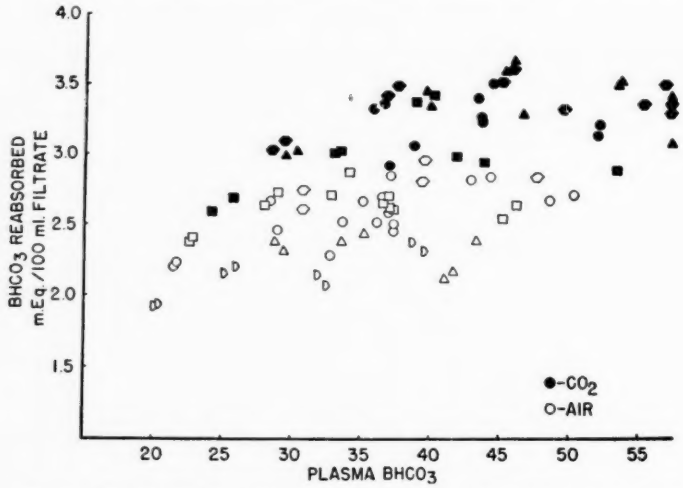

FIG. 1. THE RENAL REABSORPTION OF BICARBONATE BOUND IN THE DOG, AS A FUNCTION OF PLASMA BICARBONATE CONCENTRATION

Open circles: Respiring room air.
Solid circles: Respiring 12 per cent CO_2–88 per cent O_2.

nal tubular reabsorption of bicarbonate bound base. It is clear that the induction of respiratory acidosis was followed by a prompt and significant elevation in reabsorption of bicarbonate bound base, whether expressed in absolute terms of mEq. per min. or in relative terms of mEq. per 100 ml. glomerular filtrate. Expressed in the latter units, reabsorption on Dog A rose from an average control value of 2.48 to 3.45 during respiratory acidosis. With the resumption of breathing room air, reabsorption fell rapidly toward control values.

In order to determine the effect of CO_2 inhalation at various plasma bicarbonate levels, paired experiments were performed on each of five dogs. In these experiments, plasma bicarbonate concentration was elevated in stepwise increments from normal values to approximately 55 mEq. per liter. In the first experiment of each pair, the dog breathed room air throughout. In the second, it breathed 12 per cent CO_2–88 per cent O_2.

Data from these experiments are presented in graphical form in Figure 1. It is evident that, over the entire range of plasma bicarbonate concentration investigated, renal tubular reabsorption of bicarbonate bound base was increased by CO_2 inhalation.

Our attention was next directed toward determining which of the changes associated with respiratory acidosis was primarily effective in enhancing renal tubular reabsorption of bicarbonate bound base. The data presented thus far confirm the well-established observation that respiratory acidosis is associated with two changes in body fluid composition: elevation of pCO_2 and depression of pH (7). Stanbury and Thomson (13), and Ochwadt (14), on the basis of studies of bicarbonate excretion during hyperventilation in normal human subjects, concluded that the change in plasma pH rather than change in pCO_2 is the important stimulus effecting a change in bicarbonate reabsorption, which was decreased in their experiments. Similar conclusions were drawn by Elkinton, based on studies on hyperventilation (15) and CO_2 inhalation (16) in normal human subjects.

On the other hand, Brazeau and Gilman (17) employing controlled ventilation, as well as CO_2 inhalation in curarized dogs, varied arterial pCO_2 over a wide range (25 to 110 mm. Hg) and concluded that bicarbonate reabsorption expressed as mM per 100 ml. filtrate is practically a linear function of arterial pCO_2.

In Figure 2A, our data on bicarbonate reabsorption are plotted as a function of blood pH. It is seen that there exists a fair correlation between reabsorption of bicarbonate bound base and blood

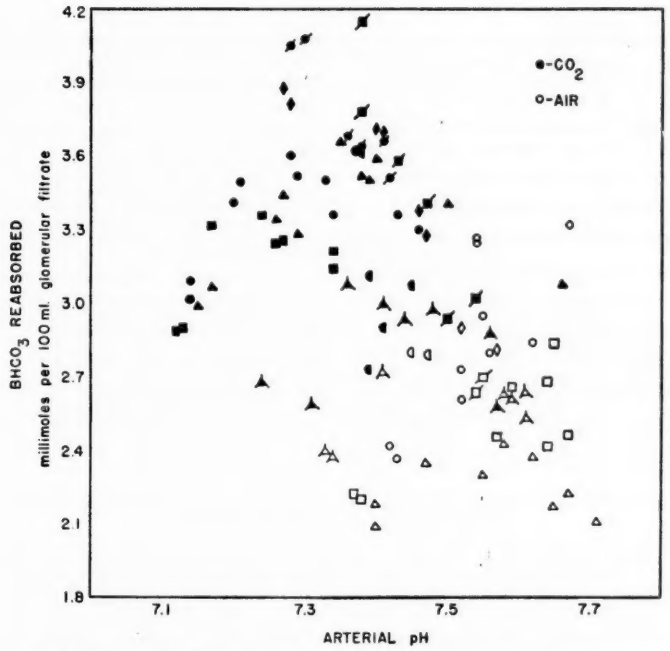

FIG. 2A. RENAL REABSORPTION OF BICARBONATE BOUND BASE, AS A FUNCTION OF ARTERIAL pH

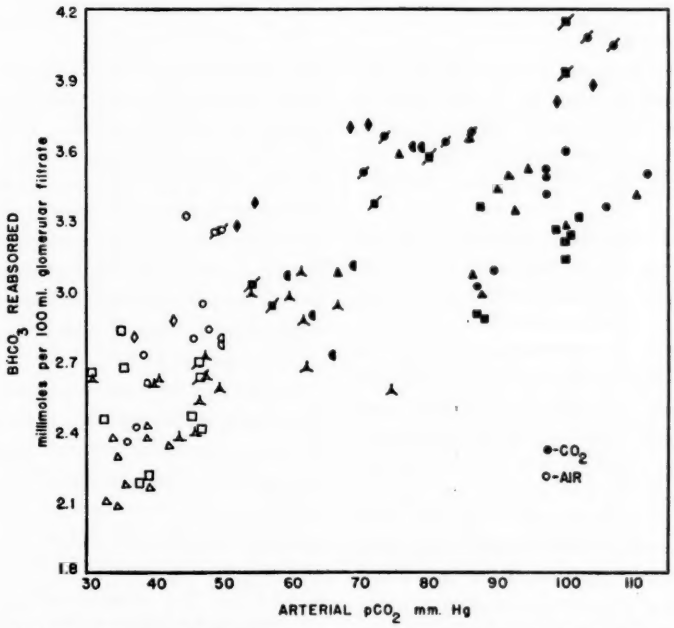

FIG. 2B. RENAL REABSORPTION OF BICARBONATE BOUND BASE, AS A FUNCTION OF ARTERIAL pCO_2

TABLE II

Experiments illustrating the effect of an elevated arterial pCO_2, with no concomitant significant change in arterial pH, on the renal reabsorption of bicarbonate bound base

Concurrent time	Glom. filt. rate	Urine flow	Plasma			Urine			$BHCO_3$			
			pH	$BHCO_3$	pCO_2	pH	$BHCO_3$	pCO_2	Filt.	Excr.	Reabsorbed	
Minutes	*ml./min.*	*ml./min.*		*mEq./L.*	*mm. Hg*		*mEq./L.*	*mm. Hg*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./100 ml. filtrate*
Experiment No. 33. Dog A—15.5 Kg.												
−65	Start infusion No. I: Creatinine 2.0 Gm./L.; $NaHCO_3$ 8.0 Gm./L. @ 5.0 ml./min.											
−60	Prime: Creatinine 1.3 Gm.; $NaHCO_3$ 4.0 Gm. Dog respiring room air											
0–15	50.9	1.64	7.49	34.8	47.3	7.97	257	116	1.86	0.42	1.44	2.82
15–30	51.0	1.64	7.50	34.9	46.3	7.97	259	116	1.87	0.43	1.44	2.82
30–45	46.9	1.51	7.49	34.2	46.3	8.00	255	106	1.69	0.38	1.31	2.80
135	Start infusion No. II: Creatinine 0.67 Gm./L.; $NaHCO_3$ 20.0 Gm./L. @ 20.0 ml./min.											
145	Prime: $NaHCO_3$ 18.0 Gm. Dog respiring 12% CO_2–88% O_2											
170–180	54.4	18.7	7.49	89.1	120.9	7.79	174	116	5.10	3.24	1.86	3.42
180–190	55.4	19.0	7.48	92.2	127.9	7.76	176	126	5.35	3.34	2.01	3.64
190–200	53.0	18.8	7.48	94.5	131.1	7.74	177	133	5.25	3.32	1.93	3.64
Experiment No. 37. Dog L—14.5 Kg.												
−55	Start infusion No. I: Creatinine 2.0 Gm./L.; $NaHCO_3$ 8.0 Gm./L. @ 5.0 ml./min.											
−45	Prime: Creatinine 1.2 Gm.; $NaHCO_3$ 4.0 Gm. Dog respiring room air											
0–15	54.9	2.69	7.47	30.4	43.2	7.48	38.2	51.5	1.75	0.10	1.65	3.01
15–30	56.3	4.11	7.47	29.4	41.9	7.38	31.5	53.8	1.74	0.13	1.61	2.86
30–45	58.2	3.97	7.45	28.8	42.5	7.46	31.0	43.7	1.76	0.12	1.64	2.82
65	Prime: $NaHCO_3$ 18.0 Gm. Dog respiring 12% CO_2–88% O_2											
80	Start infusion No. II: Creatinine 0.5 Gm./L.; $NaHCO_3$—20.0 Gm./L. @ 20 ml./min.											
100–110	48.4	15.1	7.47	69.2	98.0	7.67	115	100	3.52	1.74	1.78	3.68
110–120	52.4	16.7	7.47	71.3	101	7.67	112	97.1	3.92	1.87	2.05	3.91
120–130	51.1	18.3	7.48	73.8	102	7.65	113	104	3.96	2.06	1.90	3.72

pH. However, low blood pH in these experiments was invariably associated with an elevated arterial pCO_2; and when bicarbonate reabsorption is plotted as a function of arterial pCO_2 (Figure 2B), the correlation is at least as good and probably better.

In order to determine whether change in pH or change in pCO_2 was the stimulus effective in enhancing reabsorption of bicarbonate bound base, two further types of experiments were performed.

In the first type of experiment, the animal breathed room air initially, and sodium bicarbonate was infused at a rate just sufficient to insure frank excretion, with slight elevation of the blood pH. After several control periods, a mixture of 12 per cent CO_2–88 per cent O_2 was respired and plasma bicarbonate level was further raised by the more rapid infusion of sodium bicarbonate. The concomitant elevations of plasma pCO_2 and bicarbonate concentration were so balanced that blood pH did not change significantly from control values.

Data from two typical experiments of this variety are presented in Table II. In the first of these (Experiment 33), blood pH averaged 7.49 during the control periods. With the initiation of the CO_2 breathing and increased bicarbonate infusion, blood pH did not change significantly. However, concomitant with an increase in arterial pCO_2 from an average control value of 46.3 mm. Hg to 127 mm. Hg on CO_2 breathing, reabsorption of bicarbonate bound base rose from an average control value of 2.81 mEq. per 100 ml. glomerular filtrate to 3.57.

Similar results were obtained in Experiment 37, data from which are also presented in Table II. In this experiment control values of blood pH averaged 7.46, with no significant change with the induction of respiratory acidosis and increased infusion of bicarbonate. However, associated with an elevation of arterial pCO_2 from an average value of 42.5 mm. Hg while breathing room air, to 100 mm. Hg on breathing CO_2, reabsorption of bicarbonate bound base again rose significantly, from

2.90 mEq. per 100 ml. glomerular filtrate on room air to 3.77 on breathing CO_2.

In Table III are presented data from the second type of experiment. Here, arterial pCO_2 was held approximately constant, while blood pH was increased by stepwise increments in plasma bicarbonate concentration. In spite of a marked increase in blood pH during the course of the experiment, renal reabsorption of bicarbonate bound base remained approximately constant, presumably associated with a virtually constant arterial pCO_2.

From a consideration of these data it seems clear that increase in arterial pCO_2, rather than decrease in pH, is the stimulus effective in enhancing reabsorption of bicarbonate bound base.

DISCUSSION

The data presented thus far indicate that the renal tubular response to an acutely induced respiratory acidosis consists principally in an enhancement of the reabsorption of bicarbonate bound base, both in absolute (mEq. per min.) and relative (mEq. per 100 ml. filtrate) terms. Furthermore, the effective stimulus for this increase in reabsorption appears to be the increase in pCO_2 of the body fluids.

The mechanism of reabsorption of bicarbonate bound base proposed by Pitts and Lotspeich (12) postulated a specific transport mechanism localized to the proximal tubule, by which some 80 per cent

TABLE III

Experiments illustrating the effect of an elevation in blood pH, with virtually constant arterial pCO_2, on the renal reabsorption of bicarbonate bound base

Concurrent time	Glom. filt. rate	Urine flow	Plasma			Urine			$BHCO_3$			
			pH	$BHCO_3$	pCO_2	pH	$BHCO_3$	pCO_2	Filt.	Excr.	Reabsorbed	
Minutes	*ml./min.*	*ml./min.*		*mEq./L.*	*mm. Hg*		*mEq./L.*	*mm. Hg*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./100 ml. filtrate*
Experiment No. 18. Dog E—26.5 Kg. Breathing 12% CO_2–88% O_2 throughout												
–45	Infuse: Creatinine 3.8 Gm./L.; Mannitol 50 Gm./L. @ 5.0 ml./min.											
–44	Prime: Creatinine 2.0 Gm.											
0–15	78.6	1.70	7.14	28.7	87.0	5.78	0.95	66.1	2.37	0.0016	2.37	3.02
15–30	80.0	2.13	7.14	29.5	89.5	6.00	2.31	96.9	2.48	0.0049	2.47	3.08
30	Infuse: $NaHCO_3$ 14 Gm./L. @ 5.0 ml./min. Prime: $NaHCO_3$—6.8 Gm.											
50–65	80.3	3.46	7.21	37.7	97.1	7.55	105	123	3.16	0.36	2.80	3.49
65–80	99.0	4.12	7.20	36.9	97.1	7.52	101	126	3.82	0.42	3.41	3.41
80	Infuse: $NaHCO_3$ 28 Gm./L. @ 5.0 ml./min. Prime: $NaHCO_3$ 6.8 Gm.											
100–115	81.0	5.74	7.29	45.2	97.1	7.69	171	146	3.84	0.98	2.86	3.52
115–130	100	6.96	7.28	45.8	100	7.71	177	143	4.84	1.24	3.60	3.60
130	Infuse: $NaHCO_3$ 56 Gm./L. @ 5.0 ml./min. Prime: $NaHCO_3$ 6.8 Gm.											
150–165	108	14.3	7.34	55.4	106	7.76	187	136	6.30	2.66	3.64	3.36
165–180	89.5	12.1	7.33	57.1	112	7.75	184	136	5.36	2.23	3.13	3.50
180	Infuse: $NaHCO_3$ 84 Gm./L. @ 5.0 ml./min. Prime: $NaHCO_3$ 6.8 Gm.											
200–215	101	20.1	7.46	69.0	100	7.73	196	153	7.28	3.94	3.34	3.30
215–230	98.6	19.5	7.43	70.7	110	7.79	205	140	7.31	4.00	3.31	3.36
Experiment No. 19. Dog G—15.0 Kg. Breathing room air throughout												
–50	Infuse: Creatinine 2.0 Gm./L.; Mannitol 50 Gm./L. @ 5.0 ml./min.											
–25	Prime: Creatinine 1.0 Gm.											
0–15	39.2	4.13	7.39	19.5	33.9	6.79	3.97	26.2	0.82	0.02	0.80	2.04
15–30	35.8	2.87	7.39	19.4	33.2	6.74	3.56	26.2	0.73	0.01	0.72	2.01
47	Infuse: Creatinine 2.0 Gm./L.; $NaHCO_3$ 6.0 Gm./L. @ 5.0 ml./min.											
50	Prime: $NaHCO_3$ 4.5 Gm.											
75–90	46.9	3.47	7.50	26.0	34.2	7.77	70.5	48.9	1.28	0.24	1.04	2.20
90–105	51.8	3.20	7.52	25.1	31.9	7.83	78.2	47.2	1.37	0.25	1.12	2.15
105	Infuse: Creatinine 2.0 Gm./L.; $NaHCO_3$ 12.0 Gm./L. @ 5.0 ml./min. Prime: $NaHCO_3$ 4.5 Gm.											
125–140	56.9	6.43	7.60	32.6	34.2	7.94	120	55.0	1.95	0.77	1.18	2.07
140–155	56.2	4.93	7.60	31.8	33.6	7.96	136	61.5	1.88	0.67	1.21	2.14
160	Infuse: Creatinine 2.0 Gm./L.; $NaHCO_3$ 20.0 Gm./L. @ 5.0 ml./min. Prime: $NaHCO_3$ 4.5 Gm.											
175–190	52.3	6.80	7.65	39.6	37.2	7.98	141	61.5	2.18	0.96	1.21	2.32
190–205	50.6	5.44	7.65	38.7	36.2	8.01	158	61.5	2.06	0.86	1.20	2.38

TABLE IV

Experiment illustrating the effect of No. 6063 on the renal tubular response to respiratory acidosis

Concurrent time	Gas breathed	Glom. filt. rate	Urine flow	Plasma			Urine			$BHCO_3$			
				pH	$BHCO_3$	pCO_2	pH	$BHCO_3$	pCO_2	Filt.	Excr.	Reabsorbed	
Minutes		*ml./min.*	*ml./min.*		*mEq./L.*	*mm. Hg*		*mEq./L.*	*mm. Hg*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./100 ml. glom. filt.*
Experiment No. 21. Dog A—15.0 Kg.													
−80	Infuse: Creatinine 2.3 Gm./L.; $NaHCO_3$ 8.0 Gm./L. @ 5.0 ml./min. Prime: Creatinine 1.5 Gm.; $NaHCO_3$ 4.0 Gm.												
0–15	Room	56.8	1.80	7.43	36.1	56.2	8.00	228	93.9	2.15	0.41	1.74	3.07
15–30	Air	54.8	1.76	7.43	36.4	56.5	8.05	236	84.1	2.09	0.42	1.68	3.06
50–65		54.5	1.44	7.30	39.4	82.9	7.85	188	107.0	2.26	0.27	1.98	3.65
65–80	12%	52.3	1.37	7.25	40.4	95.0	7.83	192	116.6	2.21	0.26	1.95	3.72
80	CO_2–	Infuse: Creatinine 2.3 Gm./L.; $NaHCO_3$ 12.0 Gm./L.; No. 6063 1.0 Gm./L. @ 5.0 ml./min.											
	88%	Prime: $NaHCO_3$ 4.0 Gm.; No. 6063 0.15 Gm.											
100–115	O_2	48.7	5.50	7.24	45.1	108.8	7.70	202	165.0	2.31	1.11	1.20	2.46
115–130		50.4	5.50	7.21	44.4	114.8	7.69	203	168.2	2.35	1.12	1.24	2.45
150–165	Room	50.9	6.01	7.40	39.8	66.1	7.83	199	119.9	2.13	1.19	0.94	1.84
165–180	Air	49.6	5.33	7.39	38.5	65.9	7.84	206	119.9	2.01	1.18	0.83	1.67

of bicarbonate reabsorption was believed to be effected under isohydric conditions. The remaining 20 per cent was believed to be reabsorbed in the distal tubules by a process of ion exchange. This process could be depressed by sulfonamide compounds, presumably by virtue of their inhibition of the enzyme carbonic anhydrase, which accelerates the hydration of molecular CO_2 to carbonic acid.

However, it has recently been shown that half or more of the bicarbonate normally reabsorbed may be excreted under the influence of the carbonic anhydrase inhibitor 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (No. 6063)[4] (18). Therefore, it is our present conception that all reabsorption of bicarbonate bound base is mediated by a process of ion exchange in which the hydration of carbon dioxide to carbonic acid is an essential link.

The data in Table IV illustrate the effect of No. 6063 on the enhancement of bicarbonate reabsorption induced by breathing 12 per cent CO_2. As indicated in the first column, room air was breathed during the first and second, and also during the seventh and eighth periods. Sodium bicarbonate was infused so as to produce a moderate elevation in plasma bicarbonate concentration. Between the fourth and fifth periods, a priming dose of No. 6063 was given, and this was followed by a sustaining infusion containing No. 6063.

As may be seen from the last two columns of this table, the inhalation of 12 per cent CO_2 elicited the customary increase in reabsorption of bicarbonate bound base. Under these conditions, the infusion of No. 6063 resulted in a reduction in reabsorption of bicarbonate to values below those of the control periods. However, the further significant reduction of bicarbonate reabsorption which accompanied the resumption of breathing room air indicates that the elevation in pCO_2 of the body fluids was still effective in enhancing bicarbonate

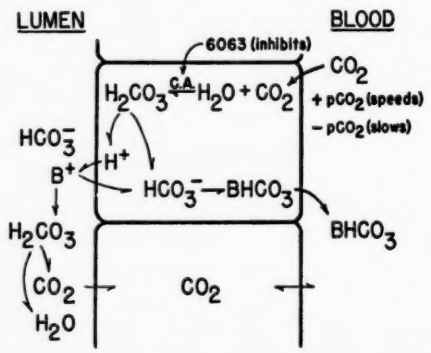

FIG. 3. PROPOSED SCHEMA OF THE PROCESSES INVOLVED IN THE REABSORPTION OF BICARBONATE BOUND BASE BY THE RENAL TUBULES

[4] We are indebted to the American Cyanamid Company for a supply of No. 6063.

reabsorption despite the inhibition of a considerable amount of carbonic anhydrase activity.

These and other data from our laboratory and from the laboratories of others (17, 19) can be explained in terms of the schema presented in Figure 3. Carbon dioxide is hydrated to carbonic acid within the tubular cells. This carbonic acid in turn dissociates to yield hydrogen ions which are exchanged for base bound by bicarbonate in the tubular urine. Carbonic anhydrase accelerates the hydration of carbon dioxide and increases the available supply of hydrogen ions. The action of No. 6063 in blocking the enzymatically facilitated hydration process reduces reabsorption of base. However, reabsorption continues, albeit at a reduced rate, for hydration occurs even though this enzyme is inactivated to a considerable degree. An increase in pCO_2 speeds the hydration process and consequently increases the reabsorption of base. A decrease in pCO_2 slows the hydration process and reduces reabsorption of base. According to this schema, the bicarbonate ion which accompanies the base into the peritubular blood is not that which accompanied it from plasma into the glomerular filtrate. This latter bicarbonate ion, first transformed into carbonic acid, dehydrates in part to form CO_2 and water in the tubular lumen. The CO_2 then diffuses back into the renal venous blood stream. Hence, in a strict sense, one should not speak of reabsorption of bicarbonate ion; rather, one must consider the reabsorption of the base bound by bicarbonate ion. How energy is cycled into this mechanism, to bring about the exchange of hydrogen ions for base, is beyond our present comprehension of the problem.

SUMMARY AND CONCLUSIONS

In normal dogs receiving infusions of sodium bicarbonate, acute respiratory acidosis induced by breathing 12 per cent CO_2 results in a significant increase in the rate of reabsorption of bicarbonate bound base, whether expressed in absolute or relative terms.

In acute respiratory acidosis, a change in pCO_2 of the body fluids is the effective stimulus in enhancing the reabsorption of bicarbonate bound base. A change in pH does not *per se* affect this reabsorptive mechanism.

The hypothesis proposed to account for these observations postulates a mechanism involving exchange of hydrogen ions for basic ions, operating throughout the renal tubule. This mechanism, enzymatically facilitated by carbonic anhydrase, is depressed by carbonic anhydrase inhibitors such as No. 6063.

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STUDIES ON THE RENAL CONCENTRATING AND DILUTING MECHANISMS IN THE PREMATURE INFANT [1, 2]

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It is known that young premature infants are unable to concentrate urine to the same degree as adults. The following hypotheses have been advanced to explain this difference: (a) A lowered production of antidiuretic hormone (1); (b) a reduced response of the distal renal tubule (end-organ) to antidiuretic hormone (2); (c) a decreased rate of solute excretion secondary to a lower rate of glomerular filtration (3).

Heller and Zaimis found that there is a lower concentration of antidiuretic hormone in the pituitary body of newborn infants than in adults, when compared on a body weight basis (1). Yet these authors believe that in spite of this smaller amount of hormone in the pituitary of the newborn infant, there is still a sufficient quantity of the hormone to produce a maximally concentrated urine, if the hormone is released and if the renal tubule of the newborn infant is responsive. Heller (2) also showed that amounts of pituitary extract, which produced a pronounced inhibition of water diuresis in adults, had only a slight and fleeting effect on the kidney of newborn infants. In comparing the response of three premature infants with that of two adults to intravenously administered pitressin during water diuresis, it was found (4) that the extent and duration of the antidiuretic response was less in the premature infant. The third hypothesis, the effect of solute excretion, can be tested by noting the changes in urine osmolarity in response to solute loading. If the lower concentrating capacity during hydropenia were solely a reflection of the lower solute load in the premature infant, then a rise in the osmolar concentration should occur with loading. On the other hand, if the response to loading were similar to the adult but of lesser magnitude, it would suggest that the lower concentrating capacity was due to a deficient tubular response. The experiments reported here were planned to test this hypothesis.

METHODS

The subjects of these studies were four premature infants weighing approximately 1500 grams but varying in ages from 5 to 23 days. To induce hydropenia they were deprived of water and formula for 12 to 18 hours. Urine aliquots were obtained to determine the maximum urine osmolarity during water deprivation.

Mannitol was used as the loading substance and was given in a 25 per cent solution intravenously. During the first 10 minutes, 180 to 200 milliosmoles per 1.73 M^2 were given and this was followed by a maintenance infusion of 1 per cent of the total priming dose per minute. Fifteen to 20 minutes were allowed for equilibration of mannitol; urine was collected under oil for three or more 10-minute periods. Blood samples were drawn before loading, after equilibration, and after the experiments were completed. The osmotic pressure, sodium, chloride and potassium of the urine and blood were determined. Osmotic pressure was determined with the Hill-Baldes apparatus (5, 6). Sodium and potassium were determined by a Perkin-Elmer flame photometer Model 52-A, using an internal lithium standard, and chloride by the method of Sendroy, modified by Van Slyke and Hiller (7). Endogenous creatinine was determined by the method of Hare and Hare (8), inulin by the method of Hubbard and Loomis (9), using the acid mixture for hydrolysis of inulin as modified by Harrison (10). These experiments were repeated on three of the four premature infants after intervals of 5 to 20 days.

RESULTS

Table I presents the data obtained with mannitol diuresis during hydropenia in four premature infants. The osmotic U/P ratio was estimated in these infants prior to mannitol loading. The values ranged from 1.75 to 2.31 with an average value of 2.10. In a group of 12 other premature

[1] Read in part before the 23rd Annual Meeting of the Society for Pediatric Research, Atlantic City, New Jersey, May, 1953.

[2] Aided by grants from the Mead Johnson Company, Evansville, Indiana, and the U. S. Public Health Service.

TABLE I

*Osmotic diuresis in hydropenic premature infants**

Patient	V *cc./min./ 1.73 M²*	Us *mOsm./ L.*	P *mOsm./ L.*	C *cc./min./1.73 M²* Cr	C *cc./min./1.73 M²* I	Osmotic U/P ratio	Creatinine U/P ratio	UosmV/Posm *cc./min./ 1.73 M²*	TcH_2O *cc./min./ 1.73 M²*	*MicroEq./min./1.73 M²* Na	K	Cl
B_I	0.59	608	276	43.0		2.20	76.0	1.30	0.71	20	50	25
Age 15 days	- - -

have to be reabsorbed from an isosmotic tubular urine to produce a concentrated urine. This quantity of water reabsorbed measured in ml. per min. has been designated by Wesson and Anslow by the symbol TcH_2O.[3] At high urine flows, in the presence of varying osmolar clearances the TcH_2O appears to be a relatively constant value for each patient. (Patient P. did not show a significant TcH_2O. The urine osmolarity of this infant during loading was isosmotic with the plasma osmolar concentration.) TcH_2O values above 2.32 which is approximately one half that of the mean adult value, were not obtained in any of these infants.

To determine whether or not this lowered water reabsorption was due to a deficiency in ADH production or insensitivity of tubular response to ADH, the following experiment was performed. After collecting urine for three periods during mannitol loading in Patient L., pitressin was given as a single injection intravenously and further collections made. The pitressin (given in a dosage of 24 milliunits per M^2) produced no further increase in water reabsorption (TcH_2O) or urine concentration. A slight increase in TcH_2O was noted with pitressin when this infant was younger.

Sodium excretions increased as much as 64 fold over the preliminary rates of 6 to 37 mEq. per min. per 1.73 M^2 amounting to around 24 per cent of the total osmolar excretion. Chloride excretions also rose in significant amounts. In contrast, potassium excretions showed only a four to five fold increase during mannitol loading and comprised about 4 per cent of the total osmolar excretion.

DISCUSSION

The observed fall in the urine osmolarity with mannitol loading in hydropenic premature infants is similar to the type of response seen in adults (12). The response in the premature infant differs in that the urine osmolarity starts at a lower level than it does in the adult and more closely approaches the plasma osmolar concentration during the diuresis.

It has been presumed that during osmotic loading in hydropenic states, there is a maximal ADH

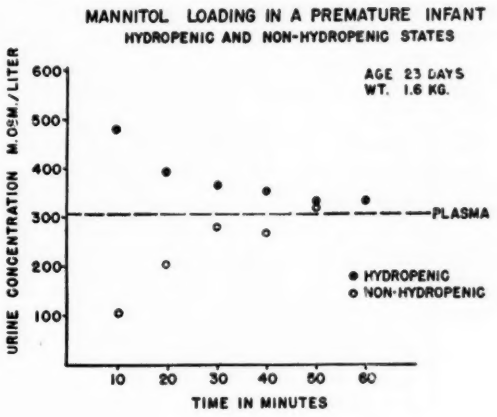

FIGURE 1

effect (13). The lack of further significant antidiuresis when pitressin was given in this study would seem to support this belief. If the decreased water reabsorption in the premature infant were due to insufficient ADH alone, the pitressin should have produced significant antidiuresis, which was not the case. It was shown (4) under other circumstances in the premature infant that there was a lessened responsiveness of the tubules to pitressin stimulation when compared to the adult. Our data, showing an inappreciable pitressin effect during hydropenia, suggest that the lowered concentrating capacity of the premature infant is probably of tubular (end-organ) origin.

In contrast to our data, Dean and McCance (14) showed that newborn infants respond with a rise in urine osmolarity when loaded with hypertonic sodium chloride or urea solutions. In order to elucidate these differences in results, the following experiment was done (Figure 1): One infant was "loaded" with mannitol during hydropenia and again four days later two hours after a 30-ml. feeding. The response to mannitol loading after the 30-ml. feeding was reversed from the usual seen during hydropenia and similar to that of the babies studied by Dean and McCance (14). Thus, under hydropenic conditions, this premature infant showed a fall in urine osmolarity with osmotic loading; and in the non-hydropenic state this same infant showed a rise in urine osmolarity.

It is of interest that the premature infant with plasma osmolar concentrations comparable to the adult has an osmolar clearance of about one third

[3] This fraction of reabsorbed water has been defined by Brodsky as "water economy" (11).

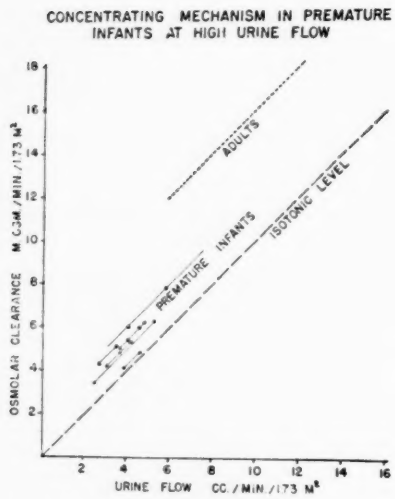

FIGURE 2

to one half the adult value (8.5 ml. per min. per 1.73 M^2 as compared to 15 to 25 ml. per min. per 1.73 M^2). This is apparently dependent upon the lower glomerular filtration rate in the premature infant. Nevertheless we can presume that his glomerular filtrate has an osmolar concentration equal to that of the adult at comparable plasma osmolar concentrations. Since the maximal osmolar concentration of his urine during dehydration is about half that of the adult (osmotic U/P ratio of about 2.10 to the adult value of 4), this lack of concentrating capacity cannot then be ascribed to a deficient filtered osmolar load, but to decreased tubular water reabsorption.

Figure 2 relates the osmolar clearance to urine volume. Although there is a variable urine flow and osmolar clearance, the quantity of water reabsorbed (TcH_2O) appears to be a relatively constant value for each infant. This value for TcH_2O at high urine flows is about one half to one third of that observed in the adult. Conversely then, at a given osmolar load these infants excrete a larger volume of water. Two of these premature infants (C and L) tested about two weeks after the initial observation were found to have a TcH_2O slightly greater than that obtained during the first observation, indicating maturing function in this relatively short period of time.

It would appear that the ultimate concentration of the urine during hydropenia is a function of the antidiuretic activity of ADH, the osmolar concentration of the tubular urine, and the responsiveness of the end-organ (tubular cells). At high urine flows, under osmotic diuresis, there is a relatively constant volume of water reabsorbed (TcH_2O). This volume of water in the premature infant is less than that of the adult but increases rapidly with aging. At low urine flows it has been suggested that the significant factor limiting water reabsorption is the osmotic concentration in the tubular fluid (15) (osmotic U/P ratio). This lowered TcH_2O and osmotic U/P ratio in the premature infant are probably dependent upon the physiologic unresponsiveness of the immature tubules.

Figure 3 demonstrates the rise in the glomerular filtration rate during loading with mannitol in one of the babies. A rise in glomerular filtration rate as measured by inulin clearance was also obtained in the two infants studied by Dean and McCance (14) when hypertonic solutions of sodium chloride and urea were administered. In contrast to the premature, the adult shows no rise in glomerular filtration rate with mannitol loading (16). This variation in response of the premature infant once again demonstrates the labile nature of his glomerular apparatus. This variability has been previously demonstrated with a fall in glomerular filtration rate during dehydration (17), in contrast to the adult response (18). The creatinine to inulin ratio, although within the normal range during the preloading phase, showed

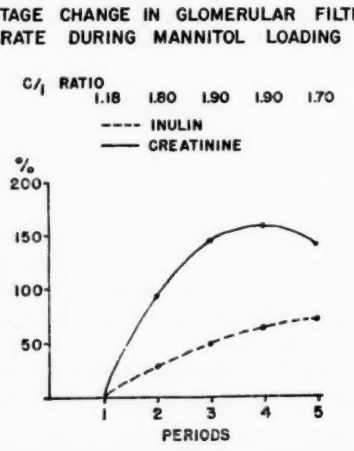

FIGURE 3

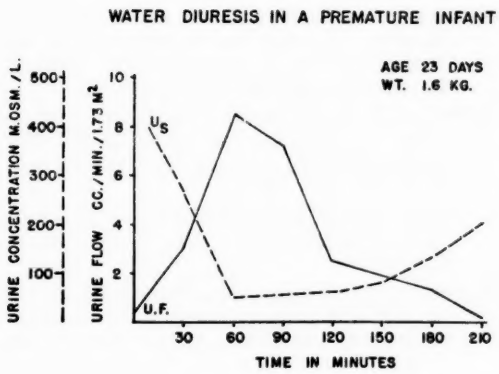

FIGURE 4

a rise to 1.90 with mannitol loading. Accepting inulin as a measure of glomerular filtration rate, the higher creatinine to inulin ratio may be interpreted as showing tubular excretion of creatinine during the loading period.

When excretions of sodium are related to the total osmolar excretions, it is found that the urinary sodium in three of the four premature infants comprises a higher percentage (average 15 per cent) of the osmolar composition than was seen in the adults (average 10 per cent) studied by Brodsky (11). The remaining infant did not show this tendency for greater sodium loss. These data suggest that the mechanism for tubular sodium reabsorption in the premature baby during osmotic diuresis is interfered with to a greater extent than in the adult. This may have important clinical implications. The chloride excretion pattern was similar to that of sodium in relation to the total osmolar excretion. Potassium excretion did not follow the patterns of sodium and chloride. These variations in electrolyte excretion are similar to those found in the adult (19).

These premature infants were also given a single water load (approximately 525 ml. per M^2). A typical response to water loading is shown in Figure 4. The maximum urine flow obtained in this patient was 8.3 ml. per min. per 1.73 M^2, while the adult under similar conditions excretes between 12 and 14 ml. per min. per 1.73 M^2. The minimal concentration of osmoles in the urine of the premature infant was about 50 milliosmoles per liter, a value comparable to that obtained in the adult. This finding of a maximal renal water excretion rate of 20 ml. per osmole for both the premature and the adult is consistent with the data reported previously (4). This lowered urine flow in the premature infant during loading with an average value of 8.2 cc. per min. per 1.73 M^2 parallels his decreased osmolar clearance. The fact that premature infant during water diuresis reaches osmolar concentrations as low as does the adult (50 mOsm. per L. or 20 ml. per mOsm.) at a time when his concentrating mechanism (TcH_2O) is less than that of the adult, suggests that these two functions may involve separate areas of tubular activity.

SUMMARY

1. During osmotic diuresis in the hydropenic state, the premature infant reabsorbs a smaller quantity of fixed water than does the adult under similar circumstances and, therefore, excretes a urine of lower osmolarity. Under the circumstances described, the constant volume of reabsorbed water measured as TcH_2O appears to be a factor limiting concentration during high urine flows. The premature infant attains a urine osmotic U/P ratio of about 2.5 during water deprivation. Adults under similar circumstances attain a value of about 4. This lowered TcH_2O and osmotic U/P ratio of the premature infant are probably dependent upon the immaturity of renal tubular function.

2. There is a variable rise in glomerular filtration rate during osmotic (mannitol) loading and a rise in the creatinine to inulin ratio.

3. Sodium is excreted at a higher percentage of the total urinary osmoles in premature infants than is found in adults during osmotic loading.

4. These same premature infants during water diuresis dilute their urine to values comparable to the adult, suggesting that the concentrating and diluting mechanisms of the kidney in the premature infant mature at different rates, and may involve different functional segments of the tubule.

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THE EFFECT OF EPINEPHRINE, INSULIN, AND HYPERTHYROIDISM ON THE RAPID INTRAVENOUS GLUCOSE TOLERANCE TEST [1]

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Although it is generally accepted that insulin accelerates the removal of glucose from the blood (1, 2), there has been disagreement regarding the effect of epinephrine on glucose utilization in normal individuals (2–5). The effect of hyperthyroidism on glucose tolerance tests has been found to be variable. The present study was undertaken to clarify the effects of epinephrine, insulin, and hyperthyroidism on the rate of removal of glucose from the blood of adult subjects by means of the rapid intravenous glucose tolerance test (6).

MATERIAL AND METHODS

All subjects had been on a diet containing at least 150 grams of carbohydrate daily for at least one week. The rapid intravenous glucose tolerance test was carried out after a 14 hour fast, and the rate of glucose disappearance from the blood was determined as described in a previous publication (6). By this method the range of blood glucose disappearance rates in normal individuals is from 3.00 to 4.84 per cent per minute.

A group of 21 normal males was employed in studying the effect of insulin and of epinephrine. A control test was performed on each subject. The test was performed in 17 of the group, 30 minutes following the subcutaneous administration of 0.5 mg. epinephrine. In a normal individual epinephrine produces a rise in the blood sugar which reaches a maximum within 25 minutes, following which there is a relative plateau for approximately 90 minutes corroborating previous work. In 18 of the group the test was performed immediately after the intravenous administration of 4 units of crystalline insulin. A time interval of approximately one month was allowed to elapse between tests in each subject.

Seventeen patients with uncomplicated hyperthyroidism were studied before treatment with radioactive iodine, and the studies were repeated when a remission of the hyperthyroidism had occurred. All the patients had been on a diet of 5000 calories daily for at least seven days prior to study. A standard oral glucose tolerance test (100 Gm. dose), a basal metabolic rate, and a 24-hour thyroid radioactive iodine uptake were also obtained. The oral glucose tolerance test was interpreted in light of the fasting blood sugar and the blood sugar level at 2 and 3 hours. The macro blood glucose determinations for the oral glucose tolerance test were carried out by the method of Benedict (7).

RESULTS

Epinephrine was found to produce a decrease in the blood glucose disappearance rate (Table I). In the control study, the mean disappearance rate was 3.64, and the range was 3.00 to 4.84 per cent per minute. Following administration of epinephrine, the mean of the disappearance rate was 1.24 and the range 0.83 to 1.82 per cent per minute. The mean of the differences being significant with

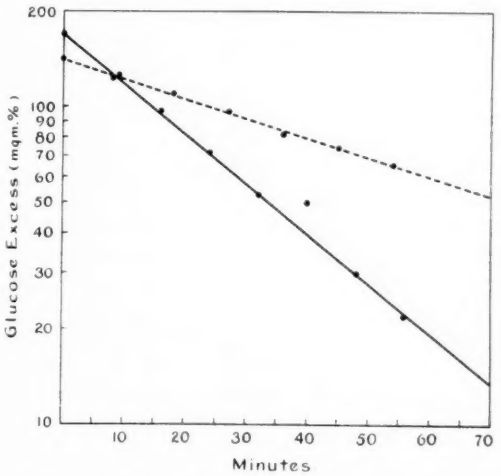

FIG. 1. THE RAPID INTRAVENOUS GLUCOSE TOLERANCE RESPONSE WITH AND WITHOUT EPINEPHRINE IN NORMAL SUBJECT, W. G.

Dotted line is with epinephrine; dark line is the control period.

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P less than 0.01. This change is illustrated in Figure 1.

Insulin produced an increase in the blood glucose disappearance rate. The mean rate in the control study was 3.59, and the range was 3.01 to 4.84 per cent per minute (Table II). After administration of insulin the mean was 7.94 and the range 4.12 to 11.90 per cent per minute. The mean of the differences between the control with and without insulin was significant with P less than 0.01. This change is graphically illustrated in Figure 2.

In moderate to severe hyperthyroidism the disappearance rate of glucose was studied in 17 patients in whom 15 were within the normal or increased range and two were slow. No correlation was found between the glucose disappearance rate and the degree of hyperthyroidism. During the hyperthyroid state, the range of the glucose disappearance rate was 2.42 to 11.17 per cent per minute (Table III). After treatment with radioactive iodine, 15 patients became euthyroid and two patients developed myxedema. Of the 15 patients with disappearance rates of glucose in the normal or increased range prior to therapy, eight became slower, five remained the same and two were slightly increased. The two patients with slow disappearance rates initially became euthyroid after treatment, the disappearance rate returning to normal in one and decreasing in the other. After treatment the range of the glucose disappearance rates was 0.31 to 8.11 per cent per minute with mean of the difference before and after treatment being significant with P less than 0.01. The change in the disappearance rate in patient W. B. is illustrated in Figure 3.

TABLE I—*A comparison of the rapid intravenous glucose tolerance test with and without epinephrine in normal men*

Patient		Fasting glucose	Glucose excess (*mg. %*) (*Time in minutes*) 0	8	9	16	18	24	27	32	36	40	45	48	54	56	Rate of removal (*% /min.*)
F. R.	a.*	91	215	150		124		114		84		67		57		36	3.35
	b.	111	161		136		134		111		100		88		79		1.12
E. E.	a	110	173	113		80		50		35		25		−3		− 5	4.84
	b.	82	193		192		181		163		151		153		140		0.62
V. K.	a.	102	165	123		103		76		62		45		33		24	3.36
	b.	101	169		158		159		138		130		113		103		0.93
A. B.	a.	97	140	109		85		64		52		39		40		21	3.44
	b.	110	113		98		80		78		75		60		54		1.28
A. Z.	a.	78	196	142		117		90		77		53		46		32	3.30
	b.	150	147		143		132		112		92		70		66		1.36
E. G.	a.	86	175	133		107		94		76		55		45		34	3.01
	b.	140	162		135		128		128		104		93		79		1.23
M. K.	a.	95	170	119		100		82		71		57		38		31	3.01
	b.	105	166		145		143		139		125		113		98		0.86
E. G.	a.	85	187	143		97		63		43		30		−1		−12	4.62
	b.	124	131		125		108		99		84		72		65		1.31
I. P.	a.	84	180	144		112		82		55		28		32		− 3	3.74
	b.	119	161		139		136		135		120		110		103		0.83
W. G.	a.	88	169	123		97		72		53		50		30		22	3.56
	b.	117	140		126		110		96		82		74		65		1.44
W. M.	a.	98	174	133		118		102		74		57		46		34	3.08
	b.	145	144		119		113		105		97		83		67		1.18
W. J.	a.	87	168	130		101		76		58		40		28		13	3.64
	b.	89	130		123		105		101		84		69		66		1.22
W. L.	a.	102	151	108		86		54		56		39		31		24	3.34
	b.	128	104		84		74		62		48		27		35		2.00
N. H.	a.	102	159	118		96		84		72		64		46		34	3.00
	b.	136	134		109		101		94		89		72		59		1.34
G. E.	a.	95	138	99		77		57		43		38		27		21	3.38
	b.	122	100		92		88		83		71		63		59		1.14
F. T.	a.	92	156	114		79		48		37		24		8		−18	4.62
	b.	115	175		162		135		116		110		87		65		1.47
L. S.	a.	92	173	126		86		58		40		28		1		−10	4.62
	b.	123	154		132		110		95		80		68		44		1.82

* a. 25 grams of glucose, b. 0.5 mg. epinephrine given 30 minutes prior to 25 grams of glucose.

TABLE II—*A comparison of the rapid intravenous glucose tolerance test with and without insulin in normal men*

Patient		Fasting glucose	Glucose excess (*mg. %*) 0	8	16	24	32	40	48	56	64	72	Rate of removal (*%/min.*)
			(*Time in minutes*)										
F. R.	a.*	91	215	150	124	114	84	67	57	36	19	19	3.35
	b.	81	185	120	74	36	8	− 9					6.30
E. E.	a.	110	173	113	80	50	35	25	−3	− 5	−15	−18	4.84
	b.	93	177	133	82	48	33	2					5.63
V. K.	a.	102	165	123	103	76	62	45	33	24	11	− 4	3.36
	b.	90	150	105	82	60	42	26					4.12
A. B.	a.	97	140	109	85	64	52	39	40	21	10	1	3.44
	b.	100	116	63	27	13	−21	−27					9.90
A. Z.	a.	78	196	142	117	90	77	53	46	32	15	2	3.30
	b.	97	157	104	49	29	− 7	−25					7.29
E. G.	a.	85	187	143	97	63	43	30	−1	−12	−12	− 7	4.62
	b.	103	185	93	33	−10	−41						9.90
M. K.	a.	95	170	119	100	82	71	57	38	31	26	15	3.01
	b.	96	167	102	55	29	−14	−38					7.37
E. G.	a.	86	175	133	107	94	76	55	45	34	27	12	3.01
	b.	95	173	103	55	30	−22	−32					7.30
I. P.	a.	84	180	144	112	82	55	28	32	− 3	− 9	− 8	3.74
	b.	87	178	107	66	36	21	−17					6.79
W. G.	a.	88	169	123	97	72	53	50	30	22	7	− 5	3.56
	b.	84	148	99	60	42	25	− 2					5.87
W. M.	a.	98	174	133	118	102	74	57	46	34	24	19	3.08
	b.	93	170	92	46	20	−22	−30					8.15
W. J.	a.	87	168	130	101	76	58	40	28	13	8	0	3.64
	b.	100	145	70	31	−26	−46	−40					9.49
W. L.	a.	102	151	108	86	54	56	39	31	24	18	15	3.34
	b.	110	123	66	31	15	0	−13					8.88
R. C.	a.	97	183	141	109	82	65	53	41	26	13	5	3.20
	b.	107	168	114	60	31	8	− 5					7.03
W. H.	a.	98	177	120	92	76	53	41	27	15	3	4	3.84
	b.	96	167	87	39	19	−18	−32					9.24
S. L.	a.	93	137	113	98	72	55	42	37	25	9	9	3.36
	b.	76	182	122	77	38	4	−10					6.30
L. S.	a.	92	173	126	86	58	40	28	1	−10	−10	−14	4.62
	b.	98	144	67	22	−22	−44	−55					11.55
M. P.	a.	98	147	110	87	70	52	41	23	20	14	0	3.30
	b.	96	142	61	21	− 8	−28	−34					11.90

* a. 25 grams of glucose. b. 4 units of regular insulin prior to 25 grams of glucose.

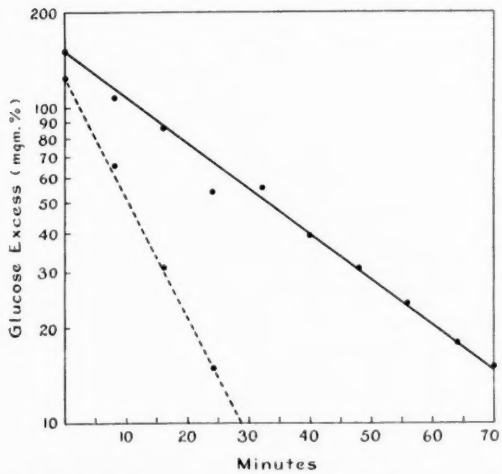

FIG. 2. A COMPARISON OF THE RAPID INTRAVENOUS GLUCOSE RESPONSE WITH AND WITHOUT INSULIN IN NORMAL SUBJECT, W. L.

Dotted line is with insulin; the dark line is during the control period.

In the 16 patients in whom the oral glucose tolerance test was done before treatment, 10 were normal and six were diabetic in type. Of the 10 patients with normal curves before therapy, two became diabetic in type after treatment; of the six patients with diabetic type tests before therapy only four became normal after treatment. No correlation was found between the glucose disappearance rate and the oral glucose tolerance test before or after treatment (Table IV).

DISCUSSION

It has been shown that in normal individuals, maintained on an adequate carbohydrate intake, the rate of disappearance of glucose from the blood during the rapid glucose tolerance test remains constant on repeated tests (6, 8, 9). It is

TABLE III

The rapid glucose tolerance test in the hyperthyroid and euthyroid states

Patient		Fasting glucose	Glucose excess (*mg. %*) 0	8	16	24	32	40	48	56	Rate of removal (*%/min.*)
			(*Time in minutes*)								
W. B.	H.*	113	119	125	72	55	36	25	17	−15	5.08
	E.	85	155	113	95	75	56	39	35	25	3.28
P. E.	H.	113	164	124	89	69	47	40	25	2	3.85
	E.	93	160	117	103	62	46	35	24	1	3.89
C. F.	H.	95	175	132	121	113	85	71	63	45	2.42
	E.	95	195	140	109	75	54	35	27	3	4.20
P. M.	H.	108	150	87	42	18	2	2	2	− 9	8.59
	M.	100	202	150	118	96	82	66	50	38	2.88
M. K.	H.	115	195	141	100	73	44	35	25	−13	4.33
	E.	90	170	136	107	78	47	42	29	12	4.41
J. B.	H.	100	155	125	96	70	62	47	38	24	3.04
	E.	102	180	139	111	88	67	60	48	34	3.04
M. P.	H.	90	168	130	88	66	45	31	10	0	4.47
	M.	95	150	124	98	79	63	48	39	30	3.00
R. R.	H.	88	132	94	65	47	40	24	16	1	4.20
	E.	100	130	100	65	43	29	21	10	5	4.71
W. L.	H.	87	147	117	95	70	59	53	39	28	3.08
	E.	103	144	117	94	78	62	50	39	31	2.86
E. R.	H.	83	164	135	104	87	61	48	37	32	3.30
	E.	110	138	110	85	64	50	34	29	11	3.33
G. G.	H.	88	185	135	101	94	77	55	38	35	3.08
	E.	94	176	124	89	66	43	26	22	6	4.08
H. K.	H.	92	158	118	86	56	38	25	16	5	4.89
	E.	96	184	132	107	77	52	32	28	7	3.74
C. R.	H.	92	156	124	104	85	72	58	48	37	2.66
	E.	107	100	103	97	93	97	93	89	85	0.31
J. F.	H.	101	192	131	94	71	54	34	23	7	4.41
	E.	98	140	120	91	75	64	47	39	32	2.90
R. D. M.	H.	105	130	67	20	−19	−32	−18	−13	− 7	11.17
	E.	115	153	77	34	17	−26	−30	−28	−23	8.11
L. R. S.	H.	98	138	95	72	52	34	22	14	15	4.62
	E.	115	144	105	80	66	46	36	18	12	3.50
S. T.	H.	100	145	85	44	25	4	4	−13	−21	7.57
	E.	77	176	118	69	38	16	13	− 4	−14	6.40

* Clinical status: H. Hyperthyroid, E. Euthyroid, M. Myxedema.

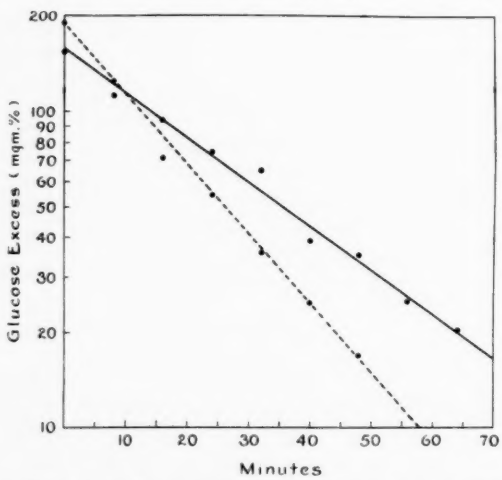

FIG. 3. A COMPARISON OF THE RAPID INTRAVENOUS GLUCOSE RESPONSE DURING THE HYPERTHYROID AND EUTHYROID STATE OF W. B.

Dotted line is during hyperthyroid state; the dark line is at euthyroid state.

known that the administration of epinephrine produces a rise in the blood sugar to a maximum level in about 25 minutes followed by a relative plateau for approximately 90 minutes, probably as a result of hepatic glycogenolysis (10). Epinephrine has also been found to prevent a normal rise in the arteriovenous difference of the blood sugar during the oral glucose tolerance test (2–4). In eviscerated rats, it also has been observed that epinephrine inhibited glucose utilization (11). The present observation of a decreased disappearance rate of glucose by epinephrine in man is probably due to the inability of the liver to assimilate a portion of the excess glucose as glycogen, and in part to a decrease in peripheral tissue utilization of glucose.

The present study is in agreement with previous

work which has demonstrated that insulin accelerates the removal of glucose from the blood (12–14). This increase in the rate of glucose removal may be a reflection of either an increased rate of entry of glucose into the cells (15), or increased phosphorylation of glucose in the hexokinase phase; both mechanisms are probably important.

A diabetic-like response to the oral glucose tolerance test in hyperthyroid subjects has led some observers to postulate an impairment in carbohydrate metabolism. However, direct studies of the action of thyroxine on tissues have revealed that there is an increased uptake of oxygen (16) as well as an increased rate of removal of glucose (17). Other studies have further indicated that the oxidation of glucose is normal or increased in hyperthyroidism (18, 19). The present study revealed that while hyperthyroid, 14 of 16 patients had either a normal or an increased rate of glucose removal from the blood during the rapid intravenous glucose tolerance test. However, five patients with normal or increased glucose disappearance rates had oral glucose tolerance curves which were interpreted as being diabetic in type.

There appeared to be little relationship between the glucose disappearance rates and the oral glucose tolerance curves in the hyperthyroid patients either before or after treatment. It may be that the intravenous glucose tolerance test is a more direct study of the effect of the thyroid hormone on accelerating the *oxidative* processes of all cells. In a similar manner, the diabetic-like curve and the excessive hyperglycemia with the standard oral glucose tolerance test may be due to the effect of the thyroid hormone on accelerating

TABLE IV

The clinical status, the rapid intravenous and oral glucose tolerance tests in the hyperthyroid and euthyroid states

Patient		% Thyroid I-131 uptake	% B.M.R.	Wt. (*lbs.*)	Rate of removal (%/*min.*)	Fasting glucose	Glucose (*mg. %*) 30	60	120	180
							(*Time in minutes*)			
W. B.	H.*	73	+38	138½	5.08	88	204	140	70	77
	E.	48	+ 0	170	3.28	93	202	140	78	68
P. D.	H.	87	+19	167	3.98	89	149	114	114	74
	E.	24	− 3	185	3.89	82	125	90	65	60
C. F.	H.	73	+30	138	2.42	117	150	145	145	103
	E.	30	− 5	141	4.20	77	132	105	77	80
M. P.	H.	71	+35	166	8.59	75	150	135	115	57
	M.	2	−35	193	2.88	85	124	93	90−	83
M. D.	H.	83	−13	130	4.53	86	140	135	94	87
	E.	9	−20	145	4.41	87	139	118	87	73
J. B.	H.	95	+37	138	3.04	76	145	159	137	78
	E.	2	− 8	155	3.04	75	138	108	88	67
P. M.	H.	81	+43	152	4.47	79	155	154	97	75
	M.	3	−26	174	3.00	63	126	158	127	103
R. R.	H.	91	+56	164	4.44	78	180	193	102	71
	E.	42	−15	167	4.71	98	114	84	77	102
W. L.	H.	54	+23	202	3.08	76	172	193	152	100
	E.	39	+ 7	203	2.90	112	215	233	170	108
E. R.	H.	6	+ 9	145	3.30	60	78	189	106	71
	E.	23	+ 4	155	3.33	85	123	148	98	60
G. G.	H.	71	+39	160	3.08	75	158	83	55	68
	E.	13	− 4	165	4.08	85	125	135	110	75
H. K.	H.	73	+42	120	4.89	73	202	224	122	43
	E.	52	+28	122½	3.79	85	215	247	118	56
C. R.	H.	55	+ 2	114	2.66	93	130	103	111	98
	E.	1	−22	118	0.31	94	140	153	130	172
J. F.	H.	88	+35	117	4.41	77	212	224	76	62
	E.	24	+10	145	2.90	80	180	165	79	73
R. D. M.	H.	58	+42	169	11.17	74	137	108	87	72
	E.	23	−12	192	8.11	87	146	133	109	55
L. S.	H.	53	+40	152	4.62	91	177	96	78	82
	E.	1	−20	167	3.50	78	130	107	101	71
S. T.	H.	70	+24	176	7.57	None		None		
	E.	None	+ 7	192	6.40	None		None		

* Clinical status: H. Hyperthyroid, E. Euthyroid, M. Hypothyroid.

the *transferring* mechanisms by the cells of the gastrointestinal tract. Similarly, the flat oral glucose tolerance test in hypothyroidism may indicate a slower *transport* mechanism by the cells of the gastrointestinal tract. This apparent difference may be interpreted as indicating that the rapid intravenous test and the standard oral glucose tolerance test measure different mechanisms of carbohydrate metabolism.

SUMMARY

The disappearance rate of glucose in 17 normal men during the rapid intravenous glucose tolerance test was found to decrease after the administration of epinephrine.

Insulin increased the rate of disappearance of glucose two to three fold in 18 normal men.

In 15 of 17 subjects with hyperthyroidism, the rate of glucose disappearance was found to be normal or increased. After remission of the hyperthyroidism the rate of disappearance of glucose was slower in eight, unchanged in five and slightly more rapid in two patients.

The oral glucose tolerance curves in 6 of 16 patients with hyperthyroidism were interpreted as diabetic in type. Of the six patients who had diabetic-like curves before treatment only four became normal after remission of the hyperthyroidism. Of the 10 patients with normal oral glucose tolerance curves before treatment, two became diabetic-like after therapy.

There was no correlation in the hyperthyroid group between the glucose disappearance rate and the oral glucose tolerance test before or after treatment.

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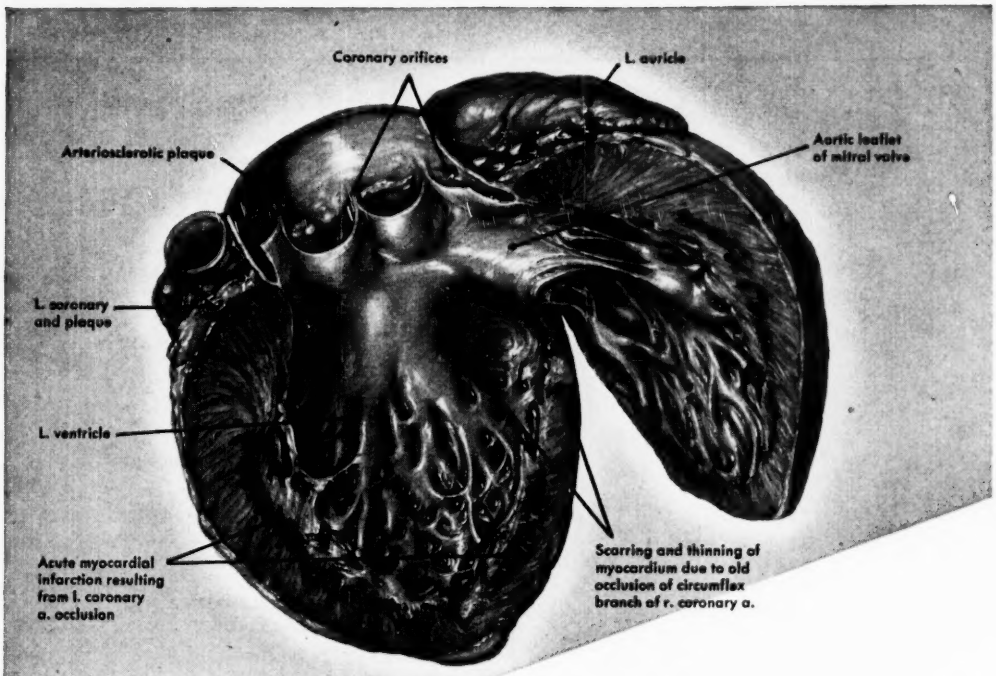
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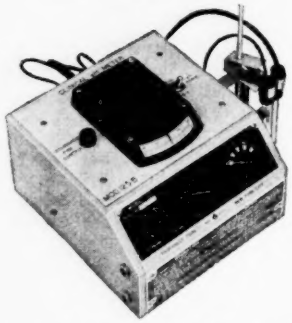

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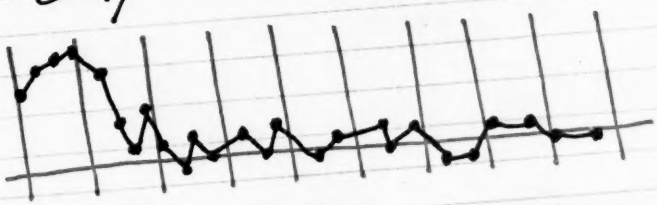

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